SOME AFFERENT AND EFFERENT CONNECTIONS OF THE CAT STRIATE CORTEX

by

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DECLARATION

The experiments described in this thesis are entirely my own work with the exception of some experiments reported in Chapters 4, 6 and 7 which were performed in collaboration with Dr. M. E. McCourt. Guidance throughout was provided by my supervisor, Dr. G. H. Henry. Part of this thesis has been published in the form of scientific papers written jointly in association with Dr. G. H. Henry and Dr. M. E. McCourt.

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PUBLICATIONS

As a result of work carried out during my tenure of a Research Scholarship in the Department of Physiology, the following papers have appeared or in the process of publication.

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Boyapati, J. and Henry, G.H. A quantitative analysis for the corticofugal and corticotectal cells of the cat.

CONTENTS

HISTORICAL BACKGROUND

THE STRIATE CORTEX

Discovery of the visual cortex

The first attempts to describe the intracranial course taken by the optic nerve appear to have been made by Galen in the second century. He suggested that vision resulted from fluids or humors flowing along fine tubes that ran from the eye to the ventricle of the brain. This view dominated for the next 1500 years and, in 1500, Leonardo Da Vinci's sketches (cf. Polyak, 1957) showed the optic nerves ending in an unrealistic series of ventricles that occupied much of the brain space.

In 1782, Gennari (cf. Polyak, 1957) made a significant discovery of local variation in cortical architecture. He was the first to observe the striated cortex in the calcarine fissures and around the poles of the occipital lobe (stripe of Gennari) but he failed to appreciate its link with the visual pathways. Only after Gratiolet's work (1856), (cf. Polyak, 1957) were the investigations of the brain directed increasingly towards the cortex. By fixing and hardening the specimen, Gratiolet was able to tease out the optic radiations and he believed, but failed to prove, that they were linked to the optic nerve. He traced the optic radiations and showed that they bypassed the lateral ventricle and proceeded on into the occipital lobe where they terminated in the striped cortex, which had earlier been discovered by Gennari and later was to be called the

striate cortex by Elliot Smith (192Ø).

Primary link with the striate cortex

Gratiolet had not been completely certain about the origin of optic radiations but this question was resolved by Minkowski (1913). He used retrograde degeneration to show that optic radiations terminating in the striate cortex actually arose from the lateral geniculate nucleus (LGN). At their point of termination in the cortex, the Marchi degeneration method showed that the geniculo-striate fibres take an oblique course through the infragranular layers and appear to terminate within the stria of Gennari (Cajal, 1922; Polyak, 1957).

Cortical lamination

At the beginning of the 2Øth century, there was a great advancement in histological methods with the introduction of Nissl and Golgi stains. These methods permitted the study of the fine structures of the visual cortex.

Brodmann (19Ø9) first presented a detailed scheme and nomenclature of the lamination in Area 17 of the monkey, human and the cat. His cortical layering was based on the embryological pictures of the cortex. In the embryo, six layers were identified in the cortex and, from the differences in cell bodies revealed in Nissl stained sections, it was possible to derive a similar layering pattern in the adult. The layers were as follows: Layer 1 consisted of the cell free zone. Layer 2, composed mainly of pyramidal cells (around 6-7 cells thick), was difficult

to separate from layer 3, which also possessed pyramidal cells. Layer 4, the granular layer, consisting principally of nonpyramidal cells, was further subdivided into 3 sublaminae in the monkey (4a,4b and 4c) and into two sublaminae in cat (4 a+b and 4c). Layer 5 consisted of big pyramids (solitary cells of Meynert) and layer 6 consisted small dark staining pyramids.

Brodmann believed that all adult cortical layers could be recognized as one of the six original embryological layers and even though laminae 2 and 3 were difficult to separate in the occipital lobe of the adult, they were obvious in the embryo. Also, despite its subdivisions in the adult, lamina 4 was treated as a single layer because it existed as a single entity in embryonic life.

At the beginning of the century also, Cajal (1911) began to use the Golgi method in the occipital cortex to examine the details of cell morphology more closely than had been possible with the Nissl stain. In the monkey, based entirely on the cell morphology, the striate cortex was subdivided by Cajal into nine cell layers. The layers were given the following descriptive titles: 1. plexiform, 2. small pyramids, 3. medium pyramids, 4. large stellate cells, 5. small stellate cells, 6. small pyramids with arcuate axons, 7. giant pyramids (cells of Meynert), 8. large pyramids with arcuate and ascending axons, 9. triangular and fusiform cells. Cajal's lamination was acceptable to students of the Golgi method but it was overshadowed by Brodmann's scheme which was based on the

more accessible Nissl stained material.

In addition to Brodmann and Cajal's cortical maps for the cat, two six layered schemes were later advanced for Area 17. These are the scheme of O'Leary (1941) modified by Lund et al. (1979) and that of Otsuka and Hassler (1962).

In Otsuka and Hassler's scheme the lamina 3/4 border was much higher than in that of O'Leary and Lund et al., who placed the upper border of lamina 4 at the level of the large pyramidal neurons at the base of lamina 3. O'Leary and Lund et al. divided lamina 4 into 4a and 4b while Ostuka and Hassler followed Brodmann in retaining the 4a+b and 4c. O'Leary introduced a sublamina 5a, comprising small pyramidal cells, which existed between the granular cells of lamina 4b and the large pyramidal cells of lamina 5b. Otsuka and Hassler (1962) did not subdivide lamina 5 but O'Leary's lamina 5a was included in their sublamina 4c.

Laminar morphology of striate cortex

Through out my studies on the cat striate cortex, I have adopted systematic lamination scheme of O'Leary (1941), with the modifications of Lund et al. (1979). The cellular basis for this scheme of lamination is as follows:

Lamina 1: Zone of almost cell free area. This layer comprises the terminal arborizations of apical dendrites of the underlying pyramidal cells and the terminals of afferent axons. There are also a small number of scattered stellate neurons with smooth or sparsely spined dendrites.

The axons of these cells distribute within the lamina or project downwards into layers 2 and 3.

Lamina 2/3: The upper border of lamina 2/3 is easily distinguished from the cell free zone and O'Leary's (1941) border pyramids are found in the lower reaches of lamina 3. Laminae 2 and 3 are difficult to separate from each other in the striate cortex and are commonly considerd together. They are composed of small to medium sized pyramids. Apart from pyramids, nonspiny and sparsely spiny stellates are also present. Most of the pyramidal neurons of lamina 2/3 project to, and receive from, ipsi- and/or contra-lateral visual cortical areas (Gilbert and Kelly, 1975; Innocenti and Fiore, 1976; Shatz, 1977; Garey and Powell, 1971).

Lamina 4: As mentioned above, the upper border of Lamina 4 is defined by the lower margin of the border pyramids in lamina 3 and the lower border, by the small arcuate pyramids in lamina 5a. A number of recent studies have confirmed that the afferent input from the thalamus terminates in lamina 4 of the visual cortex (Bullier and Henry, 1979b; Fester and LeVay, 1978; Garey and Powell, 1971; Hubel and Wiesel, 1962; 1968; LeVay and Gilbert, 1976; Tombol et al., 1975).

Stellate cells (Cajal, 1911; Lund, 1973) are common in lamina 4 while pyramidal cells, described as star pyramids (Lorente De No, 1949), are present in small numbers. Dendrites of stellate cells arise around the cell body in a radial fashion and the axon ramifies locally to form intra-cortical connection. Among stellate cells in lamina

4, a high proportion have spiny dendrites and are the recipient cells for the input from the thalamus (Davis and Sterling, 1979; White, 1979). The axons of these cells arise from the lower part of the soma and typically give off ascending collaterals as they descend towards their termination in lamina 5 or 6. On average, the spiny stellates in lamina 4a are larger than those in lamina 4b (Lund et al., 1979).

The Golgi method showed that the collaterals of axons of lamina 4b spiny stellates end in the same lamina or in lamina 4a (Lund et al., 1979) but more extensive filling of the axon collaterals with the intra-cellular injection of HRP showed that the collateral branches go higher up into lamina 3 (Gilbert and Wiesel, 1979; Martin and Whitteridge, 1984).

Star pyramidal neurons, which make up the major component of the pyramids in lamina 4, resemble spiny stellate cells except for the presence of apical dendrites which, after giving off an initial cluster of lateral branches just above to the soma, pass to lamina 2 or 1 with few, if any, side branches.

Layer 5: Cells are more sparsely distributed in lamina 5 than in other laminae. From differences in cell soma size the lamina has been subdivided into sublamina, 5a and 5b (Lund et al., 1979; O'Leary, 1941). Lamina 5a has small pyramidal neurons with arcuate axons while lamina 5b contains the largest pyramidal cells of the striate cortex.

Lamina 6: The upper border of this lamina is distinguished by the base of the large pyramids of lamina 5b and the lower border, by the white matter. The cells of lamina 6 are arranged in columns in the direction of the radiating fibres entering from the optic radiations (Henry et al., 1979; Otsuka and Hassler, 1962). Layer 6 contains both pyramidal neurons and stellate neurons with sparsely spined dendrites. In the upper half of the lamina the cells are tightly packed. The majority of cells are small to medium sized pyramidal and stellate cells. This lamina receives collaterals from the axons of LGN cells as they pass on their way to lamina 4 (Baughman and Gilbert, 1980).

Functional cell types of striate cortex

Only in the last 25 years have physiologists started to examine the functional cell types in the striate cortex of the cat. Based on the visual responses to hand held stimuli, Hubel and Wiesel (1962) first separated cells in cat striate cortex into two major groups, the simple and complex categories. Simple cells were defined as having receptive fields with spatially separate areas of ON and OFF response to stationary flashing spots or bars of light. Within these areas there is spatial summation of responses and between them, there is antagonism. To fire the cell the bar has to be set at an optimal orientation, which varies from one cell to the next.

Pettigrew et al., (1968) using moving stimuli, described simple cells as having small receptive fields, a low frequency firing rate in response to visual stimuli, a

low spontaneous activity and a preference for slowly moving stimuli. Henry and Bishop (1972) extended this description by adding that subliminal excitatory and inhibitory bands flanked the central discharge region of the simple cell receptive field.

Unlike the simple cell, the complex cell responds with composite ON-OFF discharge to stationary flashing stimuli. The areas of ON and OFF response completely overlie one another and encompass a given area to promote the idea of a uniform receptive field. There are subgroups of complex cells that have discrete areas of ON and OFF response (i.e a non-uniform receptive field) and these are distinguished from simple cells by a failure to display summation within individual response areas in the receptive field. The complex cell with a uniform receptive field is the most commonly encountered of its category in single cell recording and is usually given the unqualified designation of complex cell. Recently, the class of uniform complex cells has been subdivided into C and B categories. The C cell is the classical complex cell while the B cell has a similar uniform ON/OFF receptive field but has a smaller receptive field than the C cell, shows a preference for slowly moving stimuli and has a low spontaneous activity (Henry et al., 1983). Gilbert (1977) distinguished two types of complex cells, one which showed summation for increased stimulus length (standard complex) and the other that did not (special complex). This subdivision is not so directly linked with afferent streams as is the B/C subdivision and therefore has not been adopted in the

present study.

Hubel and Wiesel (1965) recognized a third type of cell which responded best to short optimally-oriented bars or edges and failed to respond when the stimulus was lengthened. These cells, with a preference for fore-shortened, stimuli were called hypercomplex cells. This characteristic of hypercomplex cells arises from the presence of inhibitory areas located beyond the end of the excitatory region. Hypercomplex cells, which had been thought to occur only in Areas 18 and 19 in the cat, turned out to be common, both in the cat and in the monkey, in Area 17 (Bishop and Henry, 1972; Dreher, 1972; Gilbert, 1977; Hubel and Wiesel, 1968; 1977; Kelly and Van Essen, 1974; Schiller et al., 1976; Sillito, 1977; Singer et al., 1975; Wilson and Sherman, 1976).

Hubel and Wiesel (1968) believed that hypercomplex cells were derived from complex cells but it was later shown that end zone inhibition was a common property of simple cells as well as complex cells (Bishop and Henry, 1972; Dreher, 1972). Henry (1977) then proposed a modified nomenclature which uses alphabetic titles to avoid the functional implications contained in the hierarchical terminology of Hubel and Wiesel. This nomenclature for the cell types has been adopted in the present study so that the cells have been classified in one of the following categories: S (simple) cells, C (complex) cells, B cells and the S_H, C_H and B_H titles for cells with hypercomplex properties.

Inter-relationship between the morphological and physiological cell types

In the primary visual cortex (Area 17) different classes of functional cell types are not evenly distributed throughout the depth of the grey matter. Most cells in cortical Area 17 of the cat can be physiologically classifed as S (simple) or C (complex) cells. Complex cells are found aggregated in the superficial and deep layers of the cortex whereas simple cells are more common in the middle layers (Hubel and Wiesel, 1968). Similarly, stellate cells predominate in the middle layers where afferent fibres from the lateral geniculate nucleus terminate. This parallel in the morphological and functional distributions led to the proposal that stellate cells had simple receptive fields while pyramidal cells had complex or hypercomplex receptive fields. Earlier studies seemed to support this belief and it appeared to be confirmed by Kelly and Van Essen (1974) injecting fluorescent dye intracellularly. With the study of larger samples (Gilbert and Wiesel, 1979; Lin et al., 1979; Martin and Whitteridge, 1984), however, the picture has become more complicated.

In the most recent investigations, the relationship between structure and function of particular cortical neurons has been investigated employing the intracellular injection of HRP, which has proved particularly effective and suitable for tracing the entire dendritic tree and the intracortical axonal arborization (Gilbert and Wiesel,

1979; Lin et al., 1979; Martin and Whitteridge, 1984). From these investigations, few strict relationships have emerged but some generalization may now be made. Firstly, all spiny stellate cells and most of the smooth stellate cells in lamina 4 have S type receptive fields although some smooth stellate cells were found to have C or B type of receptive fields. Secondly, most of pyramidal cells in laminae 2, 3 and 6 have S type receptive fields while those in lamina 5 are predominantly C type. Within a given lamina, therefore, there appears to be some correlation between functional and morphological categories. For example, the C cells of lamina 5 are almost certainly pyramidal, but viewed across the cortex in its entirety the differentiation breaks down and S cells depending on their lamina can be pyramidal or either type of stellate neuron.

The flow of signals through the striate cortex

The striate cortex is a structure of great complexity in which millions of cells communicate over microscopic distances. As a result, it is extremely difficult to monitor the passage of signals through such a intricate meshwork and follow their flow paths. At the moment, flow paths in the cortex are inferred by tracing the course taken by the projections of its cellular components. Ironically, the Golgi method, because of its capriciousness, has revealed the morphology of single cells that would have been obscured if neighbouring cells had also taken up the stain. In recent days, the neural tracer, horseradish peroxidase (HRP), has produced Golgi-

like pictures of cells and has brought the added advantage that HRP, which more effectively stains cellular processes, can be injected into living cells at any point from soma to terminal and then be subjected to later histological examination. Using the HRP label, it has been possible with electron microscopy (EM) to trace intra-cortical pathways and to establish the nature of synaptic contacts, both on dendrites and in axon terminals.

EM studies have shown that cells with dendritic spines receive 'excitatory' terminals (as deduced from their round vesicles and asymmetrical synaptic cleft) and have axons that make similar contacts with the next cell in the chain. The input to these cells is not exclusively excitatory, however, and inhibitory contacts are apparent on dendritic shafts and on the soma of the cell (LeVay, 1973; Somogyi et al., 1979). Nonspinous cells also receive both excitatory and inhibitory inputs on their dendrites and cell bodies but the axons of these nonspinous cells, which do not leave the cortex, terminate as inhibitory synapses and so suppress the firing rate of the recipient cell (Colonnier, 1968; Somogyi and Cowey, 1981). The inhibitory contacts on cortical cells come from interneurons while cortico-cortical and thalamo-cortical afferents have excitatory terminations. The axons of spiny stellate cells, that receive input from the LGN, do not leave the cortex and terminate in excitatory synapses (Lund et al., 1979). Primary afferent terminations also make contact on pyramidal cells which, as expected from their spinous character, also hand on excitatory signals.

Pyramidal cells are present through out all laminae although they are rare in lamina 4. Apart from the cells of lamina 5A, pyramidal cells have axons leaving the cortex although their collaterals form terminal arborizations within the cortex. Pyramidal cells of laminae 5 and 6 also give off ascending collaterals that terminate in higher laminae. It has been suggested that the collaterals from lamina 6 cells create a strong flow path for signals into lamina 4 (Gilbert and Wiesel, 1979).

From the synaptology, therefore, it appears that the flow of excitatory signals from the LGN is relayed principally through spiny stellate neurons of lamina 4. These excitatory inputs are modified by inhibitory signals coming from smooth stellate cells and in this way the firing of the relay cell is made selective for particular features of the visual stimulus. Thus, cortical cells show a preference for a given orientation, direction and velocity of movement in the stimulus. The information from cells in lamina 4 is passed to laminae 3, 5 and 6. The pyramidal cells of laminae 2/3 send axons to a variety of other cortical regions, which undertake additional processing and send reciprocal paths back to the striate cortex. Cells that lie below lamina 4, in laminae 5 and 6 send their axons principally to subcortical destinations (Gilbert and Kelly, 1975; Hollander, 1972). It is the nature of the pathways leaving lamina 6, together with their reciprocal components, that forms a central issue of the present thesis.

Extrinsic organization of the striate cortex

Of the experimental studies reported in this thesis, Chapters 1 and 3 are concerned the extrinsic pathways associated with cells in lamina 6 of the cat striate cortex. The following section, reviews the prevailing beliefs on the role of cells in lamina 6.

a) Cortico-geniculate projection:

The course taken by cortico-geniculate projection was originally identified by tracing degenerating axons revealed by the Marchi stain (Lablance, 1928; Mettler, 1935; Probst, 1902). In the cat, Probst (1902) found that corticofugal fibres pass medial to the corticopetal fibres in the optic radiations. This conclusion was based on a comparison of degenerating pathways viewed in Marchi preparations after making cortical or thalamic lesions. Later, however, it was claimed by Mettler (1935) that corticofugal and corticopetal fibres in the monkey did not take divergent courses but intermingled in the optic radiations.

More recently, the path of cortico-geniculate projection has been studied using the Nauta method (Altman, 1962; Beresford, 1961; 1962; Garey et al., 1968; Guillery, 1967; Hollander, 1970; Niimi et al., 1971; Szentagothai et al., 1966). Initially doubt was expressed by Hollander (1970) about the existence of this projection. Final confirmation of the cortico-geniculate pathway was not obtained until EM examination, after cortical lesioning,

revealed degenerating synapses in the LGN (Jones and Powell, 1969; Szentagothai et al., 1966)

The terminal branches of the cortico-geniculate pathway have been followed in Golgi preparations of cat's LGN (Guillery, 1966). Fine axons (type 1 axons of Guillery, 1966) were observed crossing the dorsal border of the LGN. These type 1 axon terminals passed through all the layers of the LGN, in contrast to the fragmented terminals that had been observed after degeneration (Guillery, 1967; Kawamura et al., 1974; Niimi, 1971). The spread of cortico-geniculate axon terminals to all layers of the LGN was also confirmed in autoradiographic studies (Hollander, 1972). Additional autoradiographic studies demonstrated that cortico-geniculate path comes from Areas 17,18 and 19 (Hollander, 1970; 1972; Updyke, 1975) and that Area 17 contributes more terminals to the LGN than Areas 18 and 19 although the autoradiographic grain density from Area 18 was heavier than that from 17. The axons from cells in Areas 17 and 18 project through all the layers of the LGN, while those from Area 19 go only to the layer C complex (Boyapati and Henry, 1984; Robson, 1983; Updyke, 1975). The injection of HRP into the LGN revealed that the pyramidal cells of layer 6 of Areas 17, 18 and 19 were exclusive in providing the source of cortico-geniculate projection (Gilbert and Kelly, 1975).

Important evidence for the possible role of the cortico-geniculate projection comes with the study of the synaptic contacts made by the cortical terminals on

principal neurons in the LGN. The cortico-geniculate fibres were identified as coming from the optic radiations from Nauta staining of degenerating terminals after cortical lesions (Garey et al., 1968; Guillery, 1967). These fibres were classed as corticofugal axons containing synaptic knobs in which the vesicles are round and the mitochondria are small and of dark appearance (RSD terminals). Such terminals are distinctive in comparison to those arising from axons in the optic tract which although they also have round vesicles, have large, pale mitochondria (RLP terminals).

b). Ipsilateral cortico-cortical projections of Area 17:

From the application of the Nauta and Gygax method to identify degenerating axons, after placing lesions in Area 17, it was demonstrated that the striate cortex was linked with areas 18, 19 and lateral wall of the suprasylvian gyrus of the same hemisphere (Hubel and Wiesel, 1965). Gilbert and Kelly (1975), using HRP as a retrograde tracer, then found, for the visual cortex of the cat, that striate neurons with cortico-cortical links reside in laminae 2 and 3. It was later demonstrated by Bullier et al., (1984), however, that cortico-cortical connections also originated from cells of laminae 5 and 6. This involvement of cells in infra-granular laminae was not great in Area 17 but increased with progression into the more lateral regions of the visual cortex.

c). Contralateral cortico-cortical projections of Area 17:

The two hemispheres are connected by a massive commissural system, the corpus callosum. The fibres linking the two occipital lobes of the cerebral hemispheres are found in the splenium of the corpus callosum (Sunderland, 194Ø) and over the years some general rules have been formulated relating to the connectivity of the visual transcallosal pathway. Thus, homotopic regions in each hemisphere are linked through the transcallosal pathway (Blakemore, 1969) and regions that do not receive an input from another region do not project to it (Jones and Powell, 1969). The strength of the homotopic component varies from area to area but for a given area it usually makes the greatest contribution (Shoumura, 1974).

Many attempts have been made to extract the transcallosal projections arising from different visual areas but an overall picture may be obtained by sectioning the corpus callosum itself. From studies using callosal sectioning, the densest degeneration was found in the lateral part of Area 17, the medial half of Area 18, the lateral part of Area 19 and in the suprasylvian gyrus (see review by Doty and Negrao, 1973). Degeneration was also found in these areas, however, even when the lesions were confined to single regions in the contralateral hemisphere. The same areas therefore, showed degeneration for lesions in Areas 17, 18 or 19 (Heath and Jones, 197Ø; 1972; Hubel and Wiesel, 1976; Jones and Powell, 1968). In most cases the heaviest part of the transcallosal projection linked

cells in those areas representing the vertical meridian in visual space. The spread of the projecting and receiving cells away from this vertical meridian representation, however, increased in the more laterally located regions of the visual cortex (Symmonds and Rosenquist, 1984)

At the laminar level, the cells contributing to the transcallosal pathway in the cat, identified from the use of the tracer, HRP, are found to reside principally in the border region between laminae 3 and 4. Contributing cells, however, occur in all laminae below lamina 1, although the contribution from lamina 5 always appears to be the smallest one. In all instances, components contributing to the transcallosal pathway have a reciprocal link of equivalent strength.

THE CLAUSTRUM

Three of the chapters in this thesis are concerned with projections going to or arising from the claustrum and it is informative to trace the growth of knowledge relating to this nucleus.

In many species, the claustrum is a voluminous nucleus of subcortical grey matter. In relation to the over-all size of the brain, however, the nucleus shows some species variation and is, for example, much smaller in man than in cat (Kappers et al., 1936). Despite its size and obvious shape, which ensured its early identification, there have been few attempts to attribute a functional role to the nucleus.

The claustrum was first called the "nucleus taeniaformis" (worm like) by Vic d'Azyr (1788-179Ø) (cf Polyak, 1957) and the name claustrum (claw shape) was introduced by Burdach (189Ø) (cf. Polyak, 1957). The claustrum is situated in the lateral half of the cerebral hemisphere, in between the insular cortex and the putamen. On its lateral border, it is separated from the island by the extreme capsule, while the external capsule runs down its medial border to separate it from the putamen. In coronal sections, the most posterior part of the claustrum has a comma shaped body, which fills out to become more like an inverted triangle in the anterior part of the nucleus.

At the microscopic level, the nucleus has neurons with stellate, polygonal or pyramidal shapes and, of these, the pyramidal shaped cells are the most common (Brockhaus, 194Ø). Functionally, the claustrum has a heterogeneous structure and has been divided into visual, somatosensory and auditory subdivisions (see below).

Little is known about the level of linkage between the functional subdivisions of the claustrum. The apparent absence of intra-nuclear axons or axon collaterals in Golgi stained material (LeVay and Sherk, 1981a) has led to the proposal that signals of different sensory modalities do not interact in the nucleus. However, such a conclusion should be treated with caution since there is often a restricted staining of axons in the Golgi material.

Embryology of the claustrum

There have been a number of conflicting ideas regarding the embryonic development of the nucleus. Meynert(1868), Wernicke(1881), Krause et al. (1906) regarded the claustrum as a derivative of the insular cortex because they believed there was a similarity in cell size and position in the two structures. Expanding on this idea, Brodmann (1909), followed by Spatz (1921) and Rose (1928), regarded the claustrum as a special sublayer which splits off from the lamina multiformis of the insular cortex. Landau (1936) completely disagreed with Brodmann's proposal since he believed that the claustrum had so many structural peculiarities that it could not be regarded as an off-shoot from the insular cortex. From his (Landau's) observations, the nucleus is situated much further from the cortex in the embryo than in the adult. The fact that the inferior part of the claustrum is intimately connected with the amygdala complex also seemed to be a point against its cortical origin. In support of his argument, Landau (1923) cited a pathological case, in which a mentally defective person lacked the insular cortex but had a well developed claustrum.

Cajal (1902) also disagreed with the concept of an off-shoot from the insular cortex, which he found to be inconsistent with the absence of vertically aligned pyramidal cells with radial or basal processes resembling those seen in the cortex. Based on this evidence, the claustrum does not appear to be of cortical origin but to

be related, together with the putamen, nucleus caudatus and amygdala, to the basal ganglia of the forebrain.

Divisions of the claustrum

i) Physiological evidence:

The unorthodox position of the claustrum between telencephalon and diencephalan originally made it difficult to decide the level on its functional homogeniety and on the origin of functional inputs to the nucleus (Azzaroni et al., 1968). Dispelling the idea of functional homogeniety, Segundo and Machne (1956) recorded multi-cellular responses throughout the claustrum while stimulating visual, auditory and somatosensory afferents. A regional organization was noted in the termination of the inputs from the different sensory modalities. Rapisarda et al. (1969) was the first to report the existence of a distinctive region of visual responsiveness within the claustrum. He showed that visually evoked potentials could be obtained in the dorso-caudal part of the claustrum and not from elsewhere in the nucleus.

ii) Anatomical evidence:

Initially, ablation methods, and later tracers, were used to prove that there are three subdivisions within the claustrum. Ablation of the visual, auditory and somatosensory cortical regions resulted in regional degenerations within the claustrum (Carman et al., 1964; Narkiewicz, 1964). More recently, injections of HRP, confined to visual cortical areas (Areas 17, 18 and 19),

revealed a restricted distribution of labelled cells were confined to the dorso-caudal segment of the claustrum (LeVay and Sherk, 1981a; Olson and Graybiel, 1980).

This historical material forms a backdrop to the studies presented in the following chapters.

SYNOPSIS OF THE STUDY

A principal aim of my study was to examine the structure and function of pathways entering and leaving lamina 6 of the striate cortex of the cat. In fulfilment of this intention, the pathways considered in chapters 1-5 all have components arising from cells in lamina 6 of the striate cortex, eventhough, in the case of the claustral link, more attention is given to the reciprocal pathway returning to the striate cortex. In the final two Chapters, 6 and 7, which deal with the transcallosal visual pathway, the cells of lamina 6 make a contribution but one which is small in comparison to that from the lamina 3/4 border region.

The format of the thesis, therefore, is as follows:

CHAPTER 1, examines the disposition of lamina 6 cells projecting to such extrinsic sites as the lateral geniculate nucleus, the claustrum and extra-striate cortex in both the ipsi- and contra-lateral hemispheres. This is a morphological study based on the retrograde transport of the axonal tracer WGA-HRP.

CHAPTER 2, presents a morphological study into the corticofugal pathway passing to the PGN and LGN from lamina 6 cells of the visual cortex. Based on the anterograde transport of HRP, this investigation examines the dimension and character of the corticofugal terminals ending in these subcortical nuclei.

CHAPTER 3, gives a account of extra-cellular recordings made from single cells in the PGN and LGN. Orthodromic responses in these cells, after electrical stimulation applied at the striate cortex, was used to obtain information on the conduction properties of corticofugal fibres. In an alternative approach, the conduction properties of corticofugal fibres were also recorded in striate neurons after antidromic stimulation was initiated in the LGN.

CHAPTER 4, contains results of a morphological examination of the cortico-claustral link. In this, the retrograde and orthograde transport of WGA-HRP, injected into the claustrum, provided information on the origin and course of reciprocal links associated with the visual segment of the claustrum. These pathways pass, not only to the striate cortex, but also to a number of extra-striate cortical regions. The quantitative evaluation of the distribution of labelled cells in these regions made it possible to determine how the strength of the claustral link with the striate cortex compared with that of other cortical regions.

An ADDENDUM, included in this chapter, describes a control experiment to observe the effect of contamination that may have involved the ectosylvian visual area (EVA) when injecting tracer into the claustrum.

CHAPTER 5, carries the study of the pathway from the claustrum to the striate cortex a stage further, by

examining the visual response properties of single striate neurons with a demonstrable trans-synaptic input from the claustrum. Responses of striate cells to an electrical stimulus applied in the claustrum provided evidence of a claustral input and the latency to these responses also gave insight into on the conduction properties of the axons carrying the signals.

CHAPTER 6, evolved from the study into the connectivity of lamina 6. The application of tracer to the trans-callosal pathway not only provided a picture of the involvement of cells in lamina 6 of the striate cortex but revealed the distribution of contributing cells throughout all laminae in a number of cortical regions. The incidence and distribution of labelled cells in these areas are presented in this chapter.

CHAPTER 7, presents a physiological study based on extra-cellular recording from striate neurons, for which there was a demonstrable trans-callosal link. The trans-callosal connection was identified by the cell's response to an electrical stimulus applied in the corpus callosum and in the contralateral hemisphere. Signal conduction times measured from these two sites provided a picture of conduction properties of trans-callosal axons and allowed a comparison with the input coming to the cell from the primary visual pathway.

CHAPTER 1

LAYERING IN LAMINA 6 OF CAT STRIATE CORTEX

INTRODUCTION

From its cellular patterns, the cerebral cortex can be arranged into six layers and, although this has been recognised for more than a century, much is yet to be learnt about the functional implications of this lamination. The connectivity of the cortex, however, gives some indication of functional possibilities for different layers. To begin with, the afferent input from the thalamus terminates predominately in lamina 4 of the striate cortex (Colonnier and Rossignol, 1969; Garey and Powell, 1971; Hubel and Wiesel, 1962; 1968) and the functional properties of cells in this layer have been studied in some detail (Hubel and Wiesel, 1962; 1968; Bullier and Henry, 1979 a&b; Mustari et al., 1982; Henry et al., 1983). The cells above and below layer 4 are mainly efferent and, in general, cells that project to a particular extrinsic site are grouped in a single lamina. Cells in the superficial laminae (lamina 2/3) connect principally with nonstriate cortical regions and to a lesser extent make commissural connection with the opposite hemisphere. In the deeper laminae 5 and 6, lamina 5 cells project to the superior colliculus (Palmer and Rosenquist, 1974) while cells in lamina 6, a central subject to the present chapter, are the source of projections to different subcortical regions such as the LGN, the claustrum and ipsi- and contralateral visual cortical areas. Prior to the present study, there was little or no information on how lamina 6 cells, projecting to these four regions, were distributed throughout the lamina.

Segregation in the distribution of cells in lamina 6 has been observed in visual association areas where it has been found that cells projecting to other cortical regions lie in the upper half of the lamina (Bullier et al., 1984). Cells with a cortical link, however, are rare in lamina 6 of the striate cortex and make up only a small part of the overall population. A second study has also mentioned the possibility that cells projecting to the claustrum reside in the middle of lamina 6 (LeVay and Sherk, 1981a) but apart from these two suggestions there is little morphological or functional evidence for segregation within lamina 6.

The AIM of the present study

The present investigation undertakes a quantitative evaluation of sublayering within lamina 6 and looks, in turn, at the laminar disposition of cells projecting to the LGN, the claustrum and to the ipsi- and contra- lateral visual cortical areas. In this way, a search was made for sublayering based on the clustering of cells that project to a particular region. The existence of such a design arrangement would provide additional evidence that cells with a common functional role are grouped together in the striate cortex. A quantitative evaluation of sublayering was undertaken within lamina 6 amongst cells projecting to the LGN, the claustrum and to the ipsi- and contra-lateral visual cortical areas. Evidence of grouping of cells would, in the future, promote the study of functional cell types and their axons inorder to obtain an indication of the functional contribution made by individual pathways.

METHODS

Cats used in the study, weighed between 2.Ø and 2.5kg and were approximately 12 months old. For the injection of tracer (5% Wheat germ agglutinin conjugated horseradish peroxidase (WGA-HRP)-Sigma), the animal was prepared with a initial injection of the sedative, Chloropromazine (Largactil, May and Bayer, Ø.2mg, i.m) and then anaesthetised with ketamine hydrochloride (Ketalar, Parke Davis, 2Ømg/kg, i.m). Craniotomies were performed, then brain surface was exposed by cutting the duramater.

Using a 1µl microsyringe, Ø.1µl of WGA-HRP was applied at different sites and the striate cortex was examined for labelled cells. Injections were made in four sites -area 19 of the ipsilateral cortex, 17/18 border area of the contralateral cortex, the visual claustrum and the LGN. Injections of each area were performed in two cats. The path taken by the microsyringe to inject WGA-HRP was varied for each area. For the claustral injection, the needle was inserted horizontally to avoid tracer contamination into surrounding visual areas (ALLS) and EVA (see chapter 3). The injections into ipsi- and contra-lateral cortex were angled to obtain the most direct approach with the least probability of contamination in the surrounding areas.

After WGA-HRP injection, each cat was recovered and allowed to survive for 48hours. The animal was anaesthetised with sodium pentobarbitol (6Ømg/kg i.p Nembutal) and perfused through the ascending aorta. Perfusion proceeded with 2ØØ ml of phosphate buffer

containing 2ml of heparin to avoid blood clots. This was followed with 1 litre of a mixture of 1.5% glutaraldehyde and 1.5% paraformaldehyde in phosphate buffer (perfused over 3Ø min and then rinsed with 2 litres of 1Ø% sucrose in phosphate buffer (at 4^{o}c).

After perfusion the brain was removed and allowed to sink in a solution of 3Ø% sucrose for 24-48 hours before sections were cut coronally at 6Øμm on a freezing microtome. Sections were then reacted, using the procedure of Mesulam (1978), with tetrāmethylbenzidine (TMB) as the chromogen.

All TMB procedures resulted in the deposition of a dark blue reaction product at sites of HRP activity. There were 4 procedural steps:

1) a brief wash in 6 changes of distilled water.

2) a pre-reaction soak for 2Ø minutes in dark in a medium that was prepared by mixing two separate solutions A and B. Solution A contained 92.5ml distilled water, 1ØØmg sodium nitro- ferricyanide and 5ml of Ø.2M acetate buffer at PH 3.3 (each 1ØØml of this buffer contained 2Øml of 1.ØM sodium acetate, 19ml of 1.ØM HCl and distilled water to bring the volume to 1ØØml). Solution B contained 5mg of ethanol and this was warmed up in order to dissolve the TMB. Solution A was added to solution B to obtain 1ØØml of medium.

This addition was made in the reaction vessel only seconds before the tissue was introduced. No solution was

older than 2 hrs. The medium was maintained with a pale reddish brown colour until the termination of pre-reaction soak to ensure the absence of chemical contamination.

3)Enzymatic reaction for 2Ø min.

This action was initiated by adding 3 ml of Ø.3% hydrogen peroxide solution per 1ØØml of the medium.

4)Stabilization for 3Ømin at Ø-4°

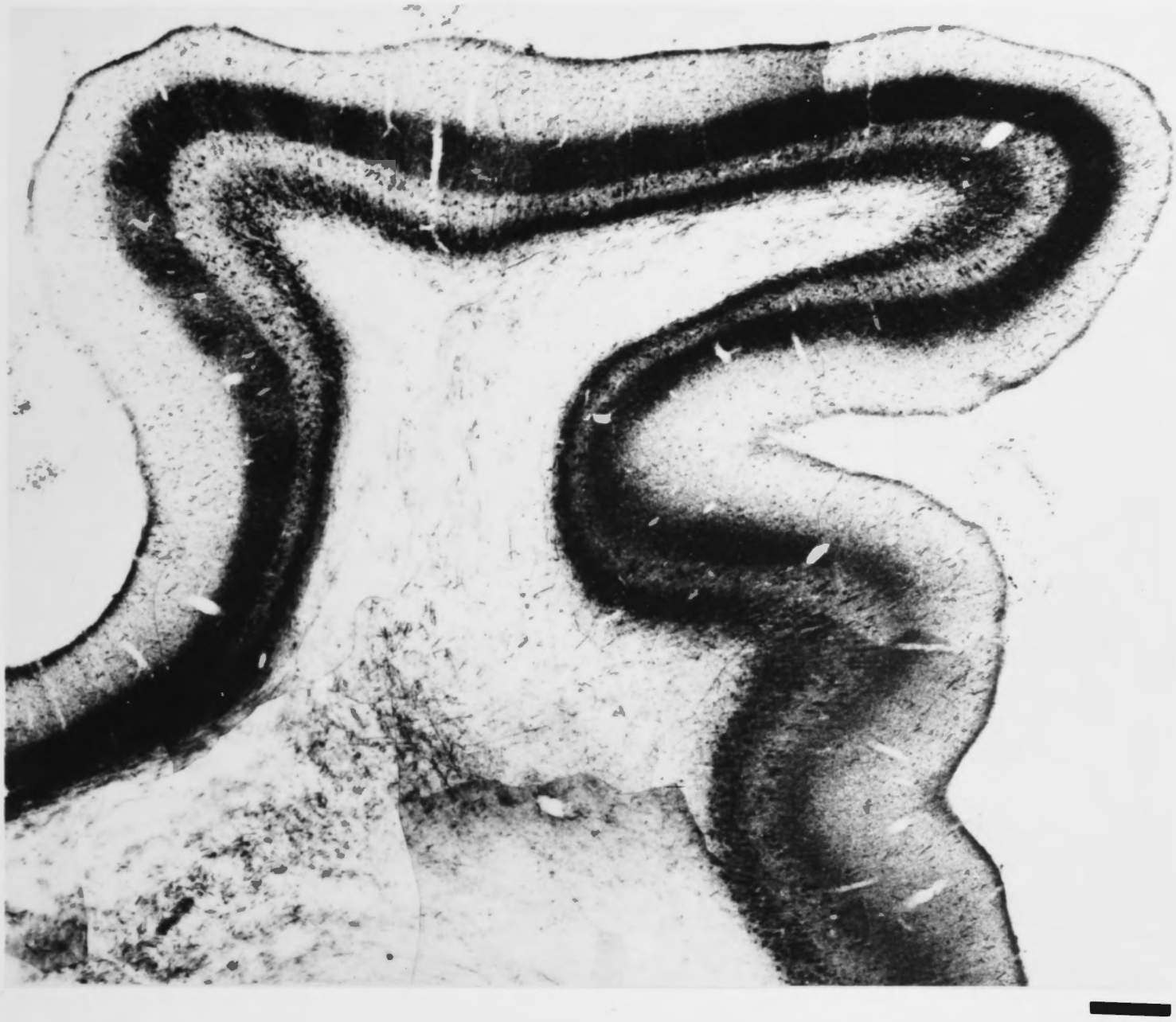
The sections were transfered without washing to a stabilization solution at Ø-4°c. Each 1ØØml of this solution contained 45 ml of distilled water, 5Øml absolute ethanol, 9 grams of sodium nitro-ferricyanide and 5ml of pH 3.3 buffer.

After stabilization, sections were washed in water, mounted onto slides, dried and coverslipped before being examined microscopically in light and dark fields. Labelled cells in appropriate regions were photographed or drawn with the aid of a drawing tube. Then coverslips were removed from representative sections and they were stained with neutral red. The counterstained sections were used to distinguish laminae and cytoarchitectonic boundaries.

The distribution of the tracer also provided a control on the effectiveness of each injection. Figures 1A and B contrast the labelling patterns resulting from the injection of WGA-HRP into the LGN (A) and the extra-striate cortex near the border of areas 18 and 19 on the ipsilateral side (B). The greatest difference between

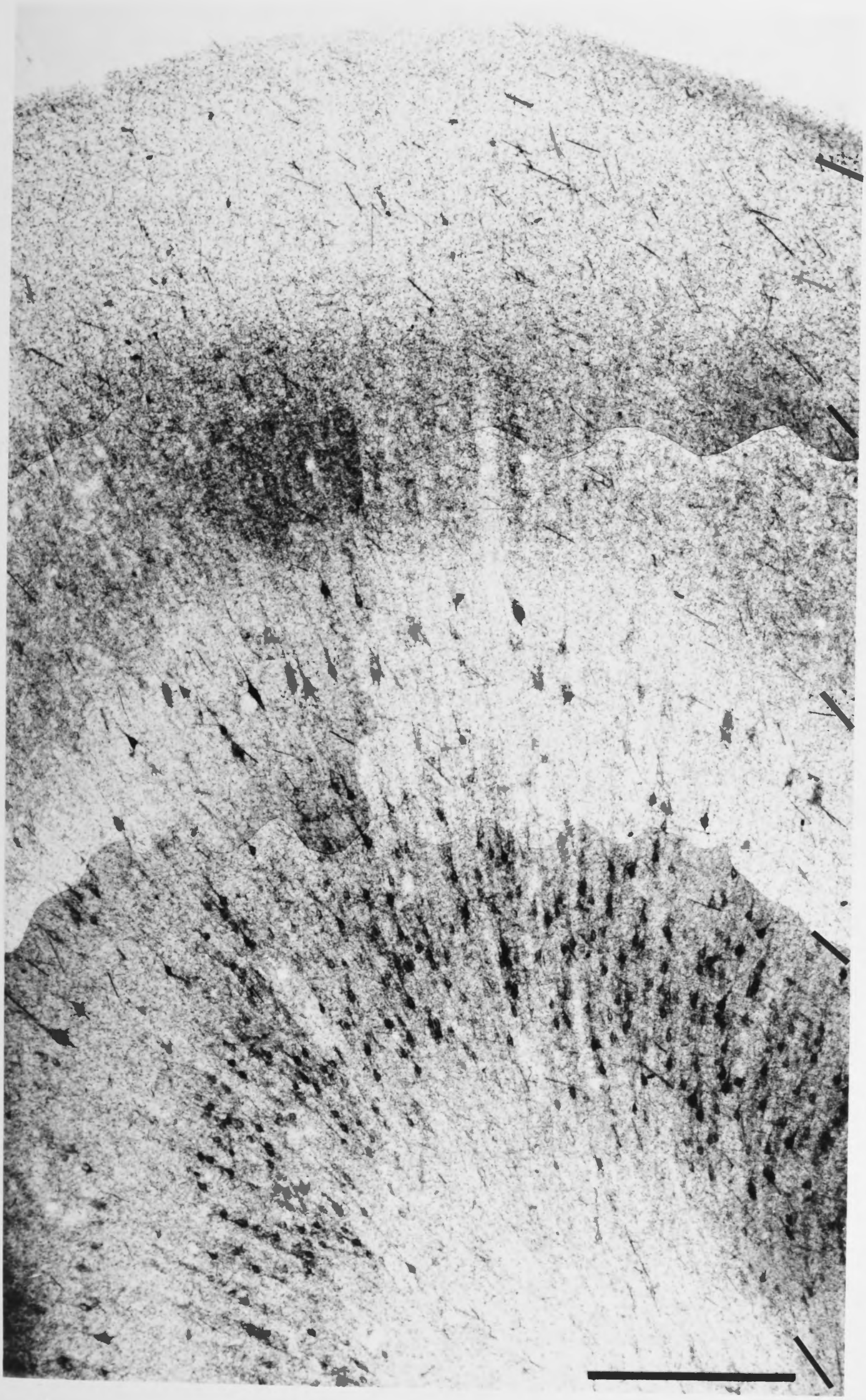
Fig. 1: A comparison of tracer distribution in cat striate cortex after injection of WGA-HRP into the LGN (A), and the visual cortex at the borders of Areas 18 and 19. Note that there are labelled cells in lamina 5 as a result of involvement of the striate-collicular pathway as it passes above the LGN. (B). Terminal filling appears as a dark meshwork and labelled cells as dark spots. Note that lamina 4 has heavy terminal labelling in A and is clear of tracer in B. The laminar borders, reconstructed under higer power closely with the staining patterns in A and B. Scale bar: 1mm.

A



B

Fig. 2: The photo-micrograph showing the density of retrogradely labelled cells in different laminae of Area 17 after the injection of WGA-HRP into the optic radiations. Large pyramids of layer 5 cell provide the land mark for marking the upper border of lamina 6. Scale bar: Ø.25mm.


1
2/3
4
5
6

these two patterns occurred in lamina 4, in which terminal labelling was heavy in Fig. 1A and absent in Fig. 1B. The absence for the terminal labelling in lamina 4 supports the belief that the cortical injection had not involved the optic radiations and that, the labelled cells in lamina 6 belonged to a true cortico-cortical projection.

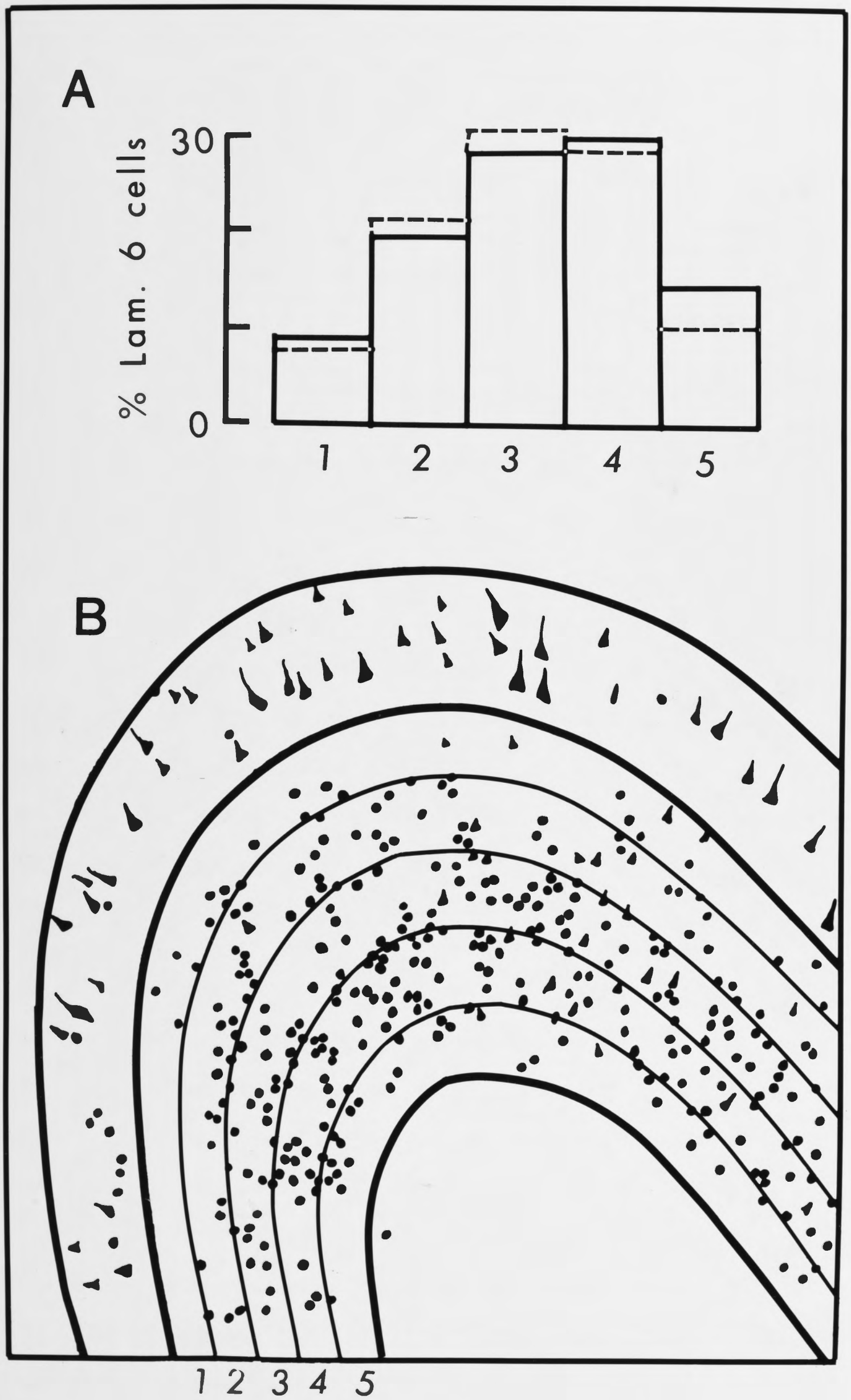
The boundaries of lamina 6 were drawn from sections stained with neutral red. As mentioned in the INTRODUCTION lamina 6 was characterized by columns or strings of cells that radiated across the lamina. The upper border was identified from the large irregularly distributed pyramidal cells of lamina 5b and the lower border by the white matter. Fig. 2 demonstrates the borders of lamina 6. The upper border is marked by big pyramids of lamina 5 (the tracer was picked up by the axons going to superior colliculus and lamina 5 cells were retrogradely filled as a result).

RESULTS

Retrogradely labelled cells in lamina 6 were plotted at x1ØØ through the drawing tube of a microscope and then, with the aid of a digitizing tablet, were stored for analysis in a computer. Lamina 6 was divided into 5 sublaminae of equal depth (Fig. 3b). The density of labelled cells were adjusted to allow for variations in the general population, assessed from Nissl stained sections. In the general population, the cell density was lowest at the bottom and highest in the middle of the lamina. Correction factors were calculated for flat and convoluted regions in the cortex since a greater density variation was found across the convoluted lamina than in regions of flat cortex.

The example in Figs. 2 and 3 is used to show how the correction factor was applied. Fig. 2 shows a photo-micrograph of labelled cells and terminals after the injection of HRP into the optic radiations. Dark spots are retrogradely filled cells and dark meshwork, the terminal labelling. From the arrangement of labelled cells (photographed in Fig. 2 and plotted in Fig. 3B) the broken line histograms in Fig. 3A have been prepared to show the nature of the raw data. The bins in Fig. 3 A represent the sublaminae of equal size shown in 3B. This raw data was adjusted by applying correction factors, assuming a lamina of uniform density, to produce the full line histogram in Fig. 3A. The extent to which each of these bins deviate from 2Ø% level (expected percentage for

Fig. 3: a. Distribution histogram (broken line- uncorrected; solid line- corrected) for bins representing sublaminae of equal size in lamina 6. b. Sublaminae drawn as schematic representation of the micro-photograph in Fig. 2. Scale bar: Ø.25mm.


A
% Lam. 6 cells
30
0
1
2
3
4
5
B
5
6
1 2 3 4 5

each of 5 equal bins in a uniform lamina) is an index of bias in the distribution.

The distributions of retrogradely labelled cells in lamina 6 were drawn up after the tracer injections into the LGN, the claustrum, the ipsilateral association cortex and the homotopic contralateral cortex. Fig. 4 shows photo-micrographs of of labelled cells in lamina 6 going to the claustrum, to the ipsilateral cortex and to the contralateral cortex and Fig. 5 the corresponding distribution histograms constructed from cell counts made in four neighbouring histological sections. The results in Fig. 5 suggest that lamina 6 cells projecting to the LGN occupy the middle of the lamina, while the cells projecting to the visual claustrum extended from the middle to the bottom of the lamina. Striate cells making cortical connections, with both ipsi- and contra-lateral hemispheres, were clustered towards the top of the lamina.

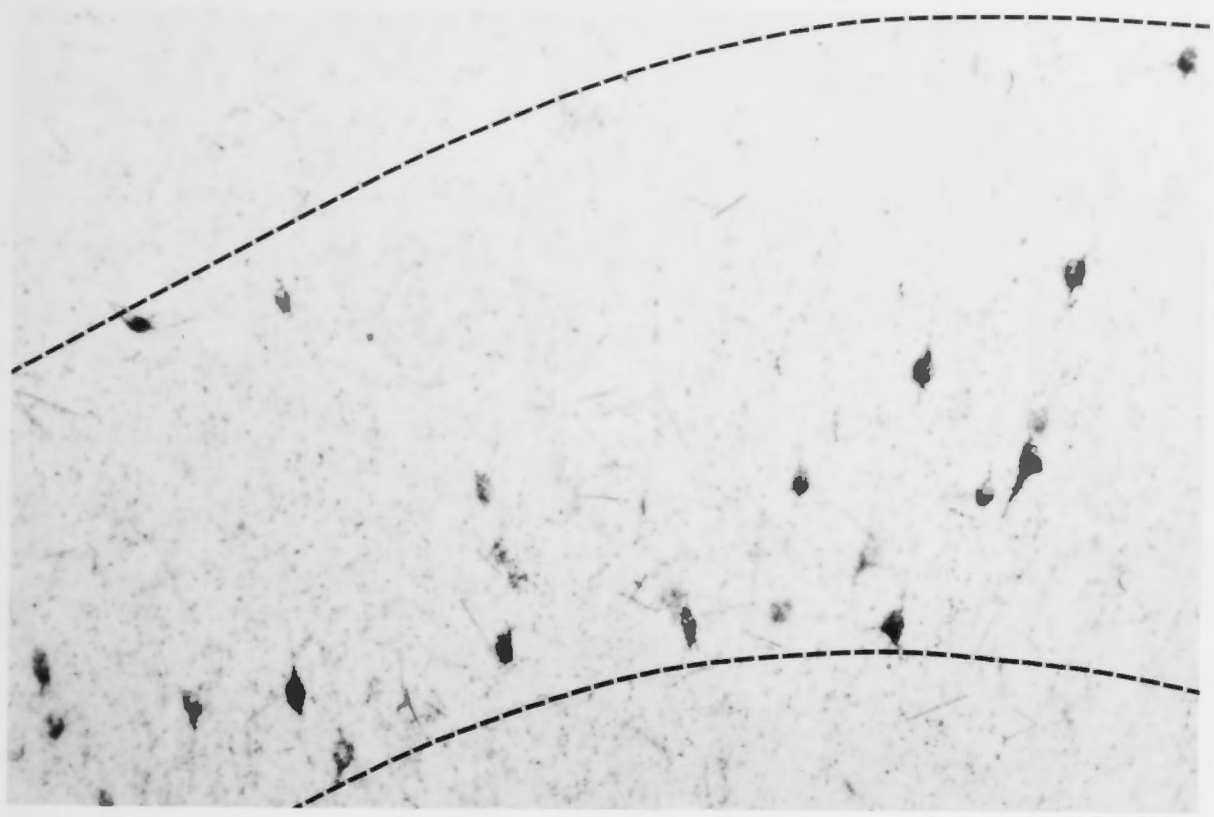
For all four populations of labelled cells the departures from uniformity across lamina 6 were statistically significant (LGN: X^2 :13.6, df:4, $p < Ø.Ø1$; claustrum: X^2 : 32.1. df:4, $p < Ø.ØØ1$; ipsilateral cortex: X^2:28.Ø, df:4, $p < Ø.ØØ1$; contralateral cortex: X^2:23.5, df:4, $p < Ø.ØØ1$). Furthermore, tested against the null hypothesis that they shared the same distribution as cells in the LGN- projection, cells of both the claustral- and cortical-projections (average of ipsi- and contra-lateral) demonstrated a significantly different sublaminar disposition (X2: 56.4, df :4, $p < Ø.ØØ1$ and X2:51.3, df:4,

$p < 0.001$, respectively). Thus, while there is obvious overlap in the histograms shown in Fig. 3, the distributions are none-the-less significantly different.

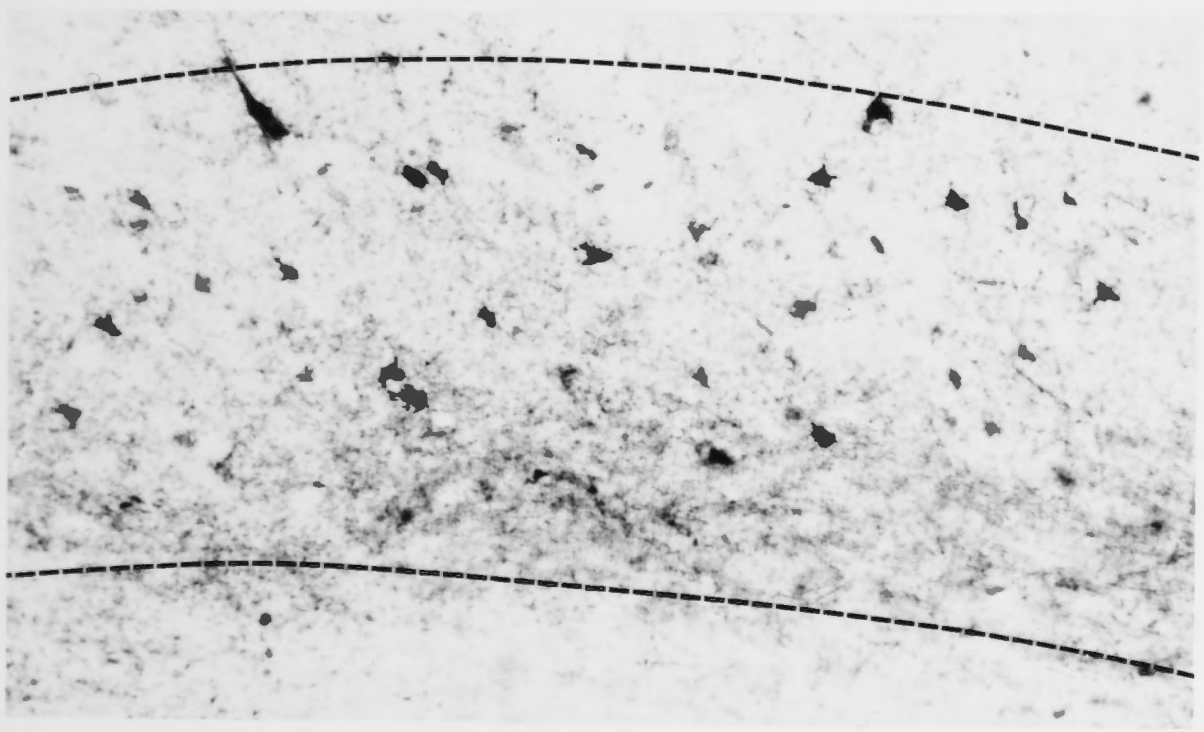
To obtain the proportion of lamina 6 cells contributing to the 4 projections, counts were made from the section with the highest incidence of labelled cells. These counts showed that 42% of cells project to the LGN, 9% to the claustrum, 13% to the ipsilateral cortex and 8% to the contralateral cortex. From these statistics it appeared that, 72% of lamina 6 cells projected to four destinations. The remainder of the population may project to other, as yet, unidentified sites or possibly they are interneurons with locally distributing axons. These figures do not allow for the possibility of efferent branching that all projecting cells were effectively labelled.

Fig. 4: Micrographs showing distributions of retrogradely labelled cells in lamina 6 of the striate cortex after the injection of tracer into A: the claustrum, B: Area 19 of the ipsilateral cortex, and C: Area 17/18 border region of the contralateral cortex.

A. TO CLAUSTRUM



B. TO IPSI LAT. CORTEX



C. TO CONTRA LAT. CORTEX

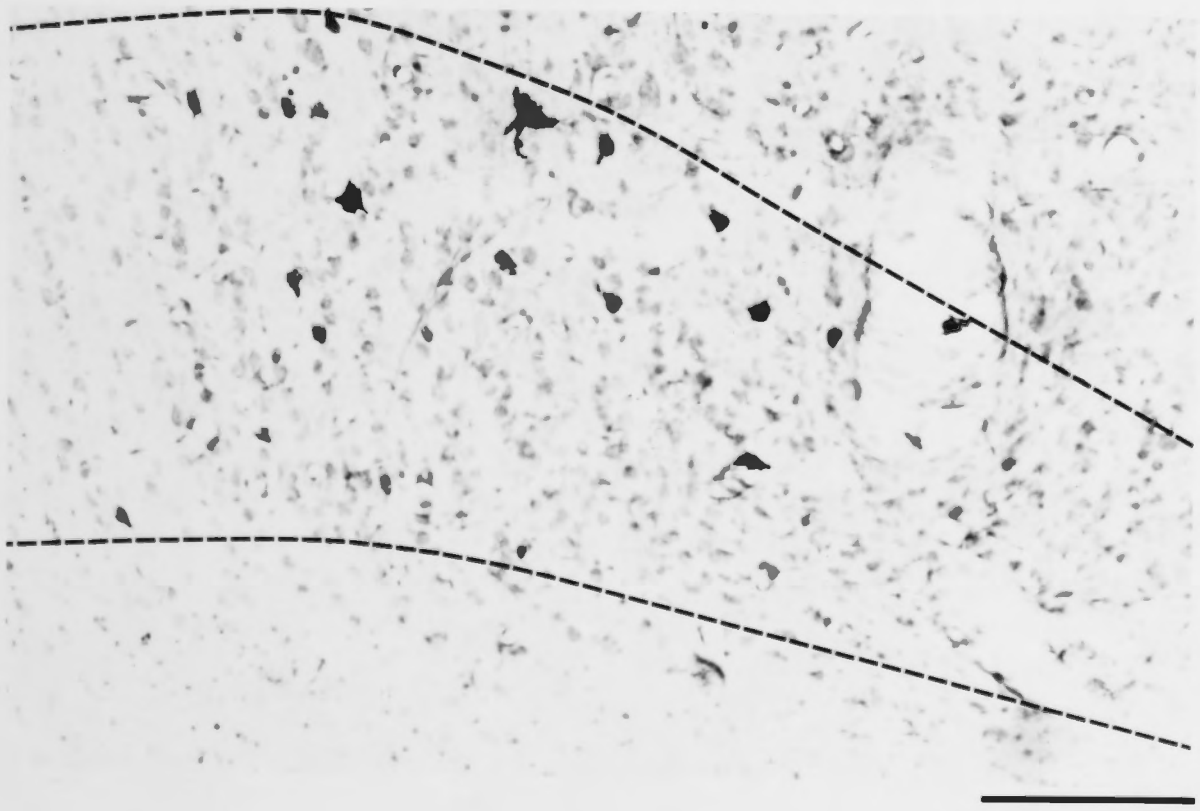
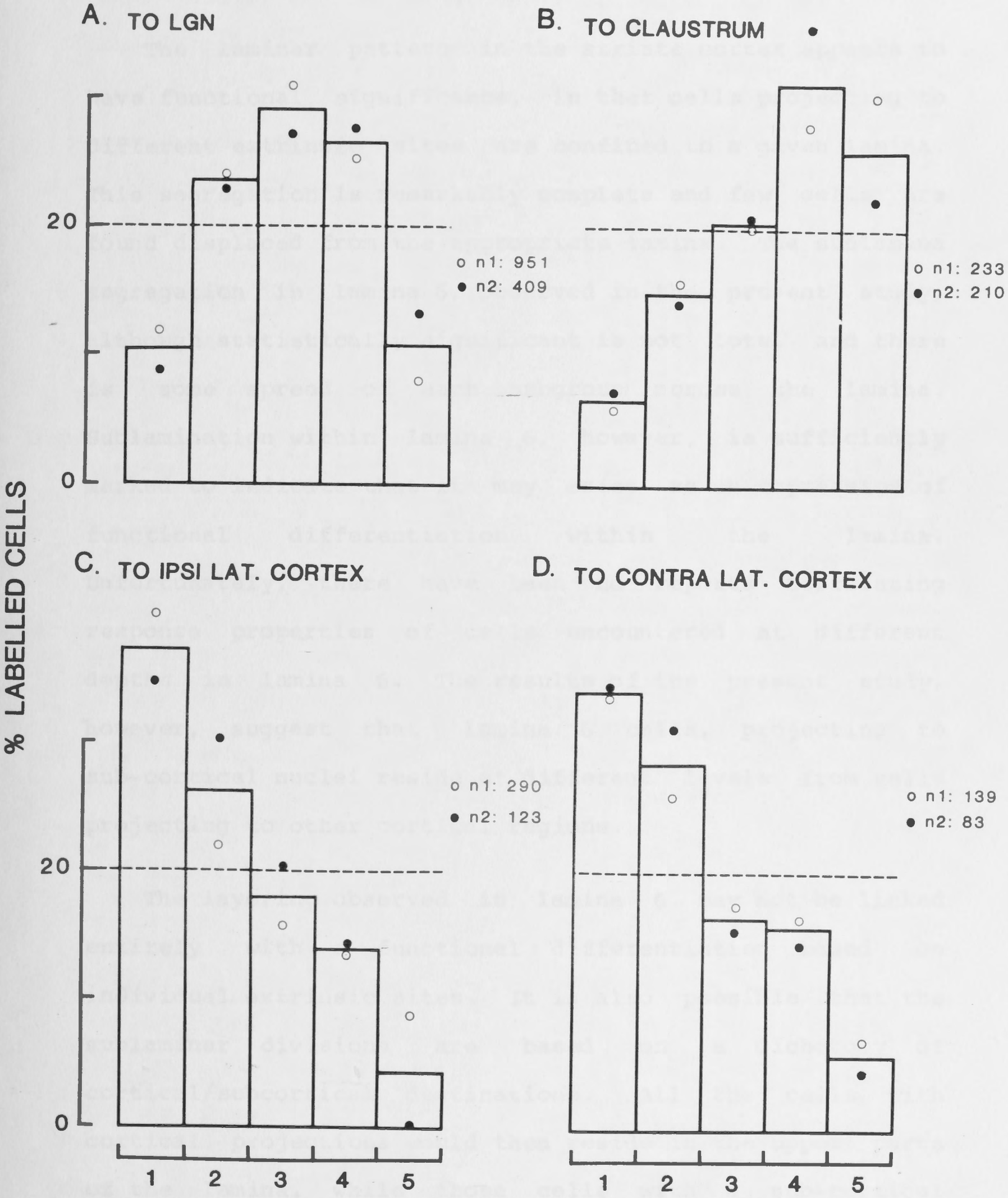


Fig. 5: Distribution histograms (corrected as in Fig. 3a) showing the percentage of labelled cells in each bin and after injection of tracer into each of the nominated sites. Bar heights are the mean of the findings from two animals. The plotted points are the results from each animal. Broken line, at 20% level, is expected for uniform distribution of labelled cells.

PROJECTIONS OF LAMINA 6



DISCUSSION

The laminar pattern in the striate cortex appears to have functional significance, in that cells projecting to different extrinsic sites are confined to a given lamina. This segregation is remarkably complete and few cells are found displaced from the appropriate lamina. The sublamina segregation in lamina 6, observed in the present study, although statistically significant is not total and there is some spread of each subgroup across the lamina. Sublamination within lamina 6, however, is sufficiently marked to indicate that it may exist as an expression of functional differentiation within the lamina. Unfortunately, there have been no reports correlating response properties of cells encountered at different depths in lamina 6. The results of the present study, however, suggest that lamina 6 cells, projecting to sub-cortical nuclei reside at different levels from cells projecting to other cortical regions.

The layering observed in lamina 6 may not be linked entirely with a functional differentiation based on individual extrinsic sites. It is also possible that the sublaminar divisions are based on a dichotomy of cortical/subcortical destinations. All the cells with cortical projections would then reside in the upper parts of the lamina, while those cells with a sub-cortical projection (to the LGN and the claustrum), would be in the lower reaches. Although there is some agreement with this design the present results, indicate that cells projecting

to the LGN spread throughout much of the lamina and overlap those projecting to extra-striate cortex and the claustrum, which lie in the upper and lower extremes, respectively. A destination-dependent distribution rather than one based on a cortical/subcortical differentiation appears to hold for lamina 6. It seems likely from this design, that information going to the cortex and the claustrum is more restricted in its range than that going to the LGN, which monitors cells from a wider expanse of the lamina.

CHAPTER 2

CORTICOFUGAL AXONS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT

INTRODUCTION

In the cat, the functional role of the corticofugal projection to the LGN and its adjacent dorsal cap, the perigeniculate nucleus (PGN), remains uncertain even though cortical cells with axons contributing to this pathway have been identified in physiological experiments (Gilbert, 1977; Harvey, 1978; 198Ø). In a sample of 27 striate neurons, identified from their antidromic responses to electrical stimulation just above the LGN, Harvey (198Ø) found 85% were simple or S cells and 15% were complex or C cells. Both these cell types are highly specific in their stimulus requirements, yet this feature is not passed back to the LGN, so that the specificity of the cortex is not reflected in the firing of LGN cells. The two cell types projecting to the LGN also differed in that the axons arising from S cells appeared to be finer than those from the C cells. Thus, the S and C cells had antidromic latencies that were, respectively, greater than and less than 2ms. This distinction was similar to the one observed in the orthodromic latencies (following cortical stimulation) for cells terminating in the LGN and PGN (Dubin and Cleland, 1977). As a result Harvey (198Ø) suggested that S cells provided the input to the LGN while the projection to the PGN came from C cells.

Anatomical investigations have given little support to the notion of a dichotomy in which the fine axons go to the LGN and the coarse to the PGN. Many of these studies (Cajal, 1911; Guillery, 1966; Polyak, 1957) were limited to

such small terminal twigs in the LGN that it was impossible to be sure of their corticofugal origin. Guillery (1966) found some axons that crossed the dorsal border of the LGN and terminated before the optic tract (type 1 axons) and concluded that these, at least, could arise from the cortex. These axons were of fine calibre and appeared to communicate either through terminal clusters of boutons or through varicosities along the length of the terminal.

Guillery (1967) further examined the termination of the corticofugal pathway in the LGN by tracing degenerating axons after lesions in the visual cortex. Fine axons were found to pass directly to each layer in the LGN but terminated with diminishing density in layers A, A_1 and C complex (Robson, 1983). Coarser fibres, rather than going to the PGN as proposed above, appeared to skirt the medial and lateral border of lamina A and terminate in the central interlaminar nucleus (CIN). Updyke (1975) also showed by autoradiographic method that cortical projections could be more pronounced into CIN than into layers of the LGN. This was particularly true for the projection from Area 18.

The AIM of the present study

This study sought a more detailed picture of the individual axon terminals of the corticofugal pathway in laminar LGN and the PGN. The axons were revealed through the anterograde transport of HRP. The enzyme was injected above the LGN to obtain effective staining of myelinated axons and also of any collaterals passing into the PGN. Labelled axons were graded according to their calibre, their destination and the morphology of their appendages.

METHODS

In preparation for the injection of HRP, one-year-old cats, weighing between 2.Ø and 3.5kg, were first sedated with chloropromozine (1mg/kg) and then anaesthetised with ketamine (2Ømg/kg, i.m). Craniotomies were performed to allow injection of Ø.1 to Ø.3µl of 2Ø% HRP (Sigma type vi) from a 5µl microsyringe with its tip positioned stereotaxically in the optic radiations (Horsley-Clarke AP 5; ML 9 and at a depth of 1Ø mm from the pial surface). The animal was allowed to survive for 8-1Øhrs (Mesulam and Mufson, 198Ø). After anaesthetising with Nembutal (5Ø mg/kg i.p.), the animal was perfused with 1.5% glutaraldehyde and 1.5% paraformaldehyde in Ø.1 M phosphate buffer. The brain was then removed and stored overnight at $4^{o}C$ in 3Ø% sucrose in buffer.

Frozen parasagittal sections, 8Ø µm thick, were cut in a sledge microtome. Sections were reacted with 3,3'-diaminogenzidine tetrahydrochloride (DAB) using the method of cobalt intensification described by Adams (1977). A dark blue to black reaction product was formed using DAB when the incubation was proceeded by cobalt chloride ($CoCl_2$) treatment. In this proceedure sections were placed in Ø.1M tris -HCl buffer (PH 7.6) for 5 minutes, moved to Ø.5% $CoCl_2$ in the same tris buffer for 1Ø minutes and carried twice more through tris and through Ø.1M phosphate buffer (PH 7.3) for 5 minutes each. Sections were then pre-incubated for 1Ø-15 minutes in DAB and then incubated for 2Ø-3Ø minutes in a hydrogen peroxide solution in

phosphate buffer at room temperature (Graham and Karnovsky, 1966). Sections were then transferred through two rinsing solutions of buffer, mounted on gelatinized slides and counterstained with neutral red. HRP filled axons were examined under oil at a magnification of x1,ØØØ and drawn with the aid of a drawing tube.

RESULTS

HRP was injected into the optic radiations of four cats at a point at least 2mm above the dorsal surface of the LGN. A diffuse halo formed around the injection site but did not ever extend into the LGN. Injections greater than 5mm from the LGN were ineffective in anterogradely filling terminals in the LGN. In each cat numerous axons were infiltrated.

Since the HRP was injected into the optic radiations it was not possible to be certain of the cell of origin. There were good indications, however, that the axons were descending and also that they came from the cortical Areas 17, 18 or 19. In contrast to axons ascending from the LGN to the cortex, the corticofugal axons tapered as they passed downwards and had a distinctive branching pattern in which terminal appendages ended in different layers of the LGN (cf. Friedlander et al., 1981). A cortical origin for the infiltrated axons was also supported by the observation that the somata of cells in laminae 5 and 6 of areas 17, 18 and 19 were retrogradely filled with HRP. It is highly probable, therefore that the axons projecting to the LGN arose from cells in lamina 6 of these cortical regions (Gilbert and Kelly, 1975).

The diameters of axons, judged to be corticofugal from the above criteria, were measured where they crossed the dorsal border of the LGN. For 29 axons the range of diameters was Ø.5 and 1.7µm. At times axons between Ø.5 and 1.Øµm were difficult to trace since they came close to

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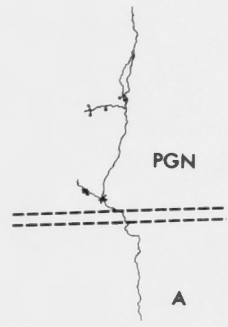
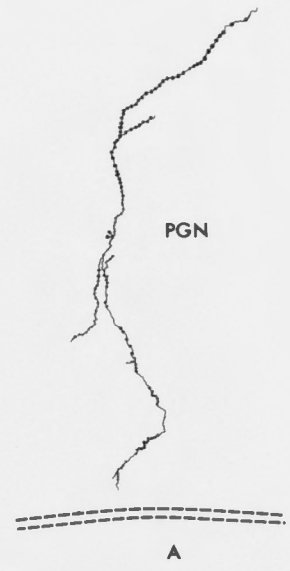
I

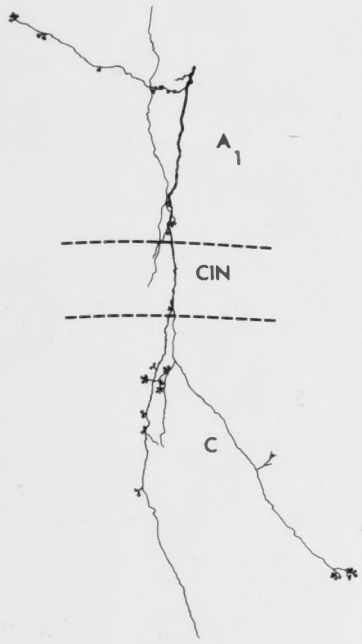
III



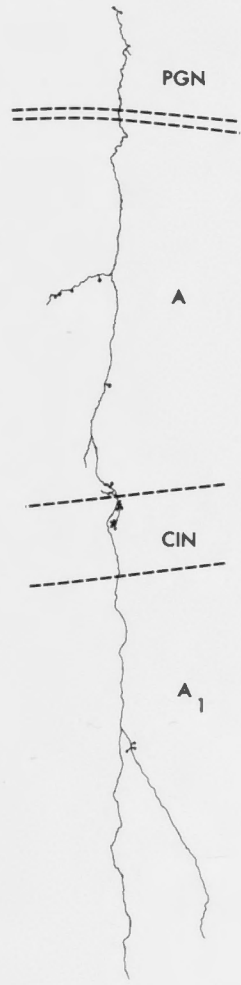
V



II



IV



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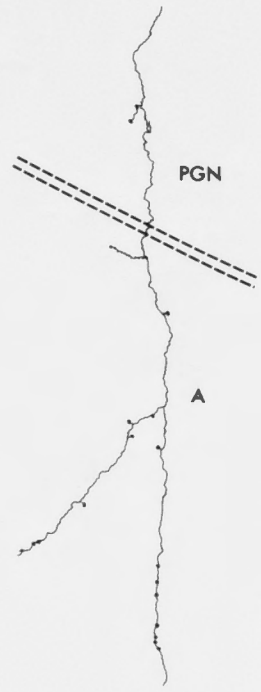

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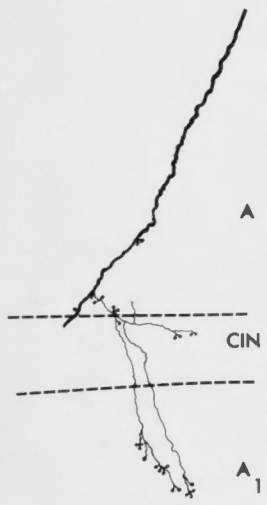

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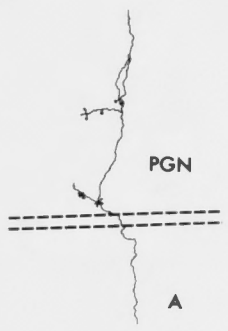

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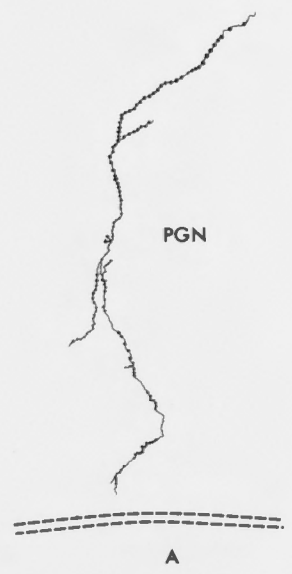

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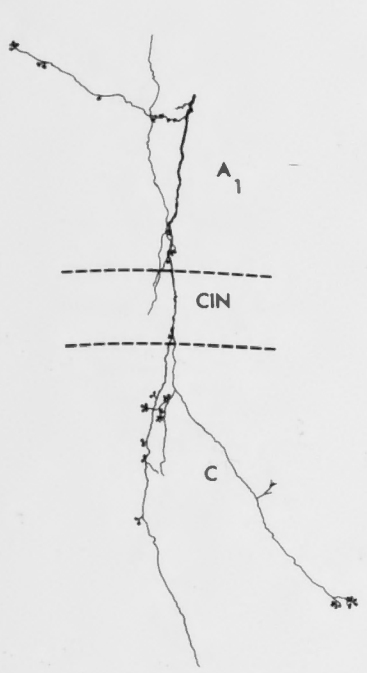

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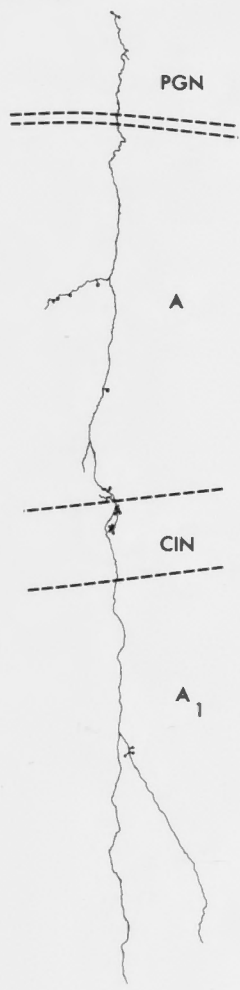

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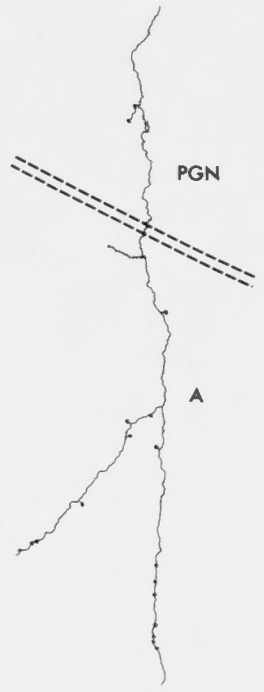
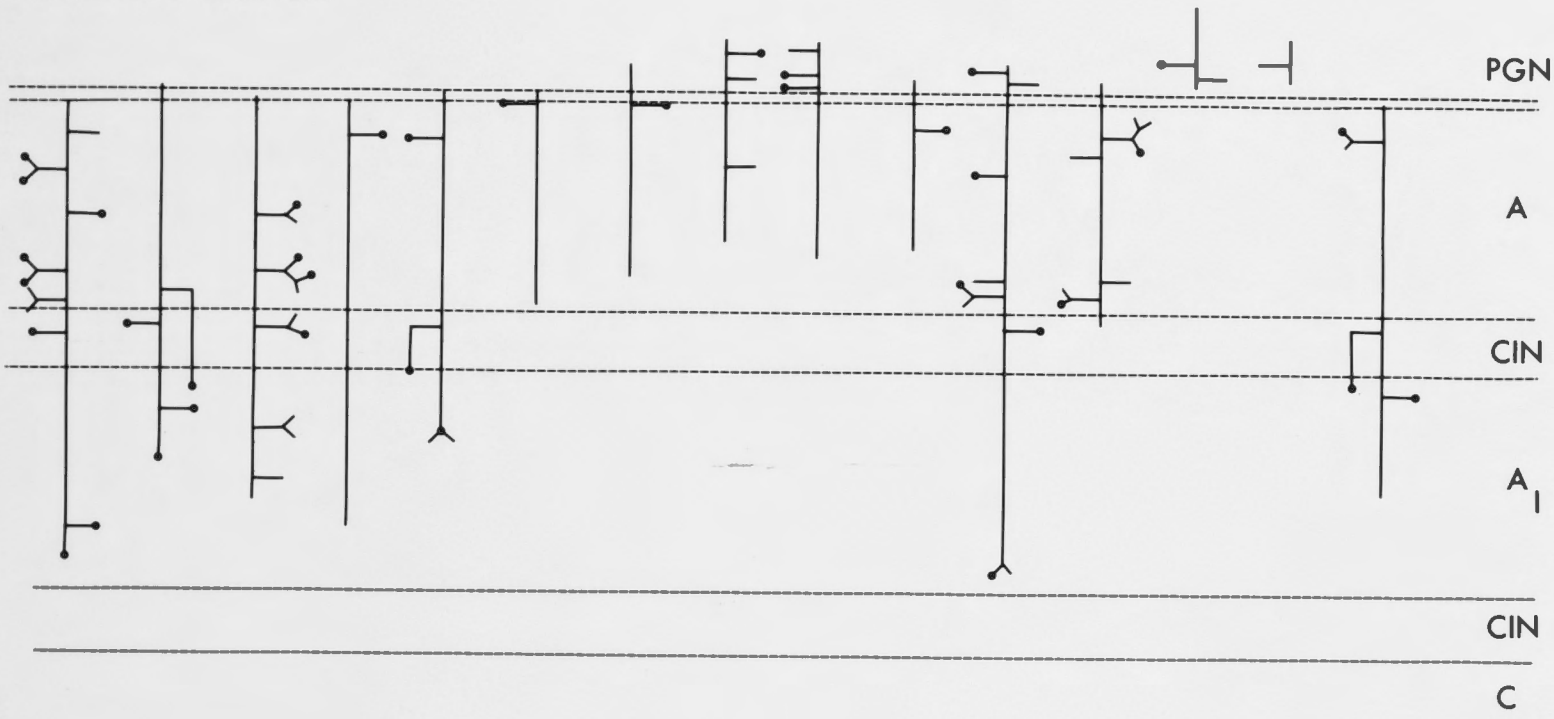


THIN TERMINALS

WITH END CLUSTERS



WITH VARICOSITIES

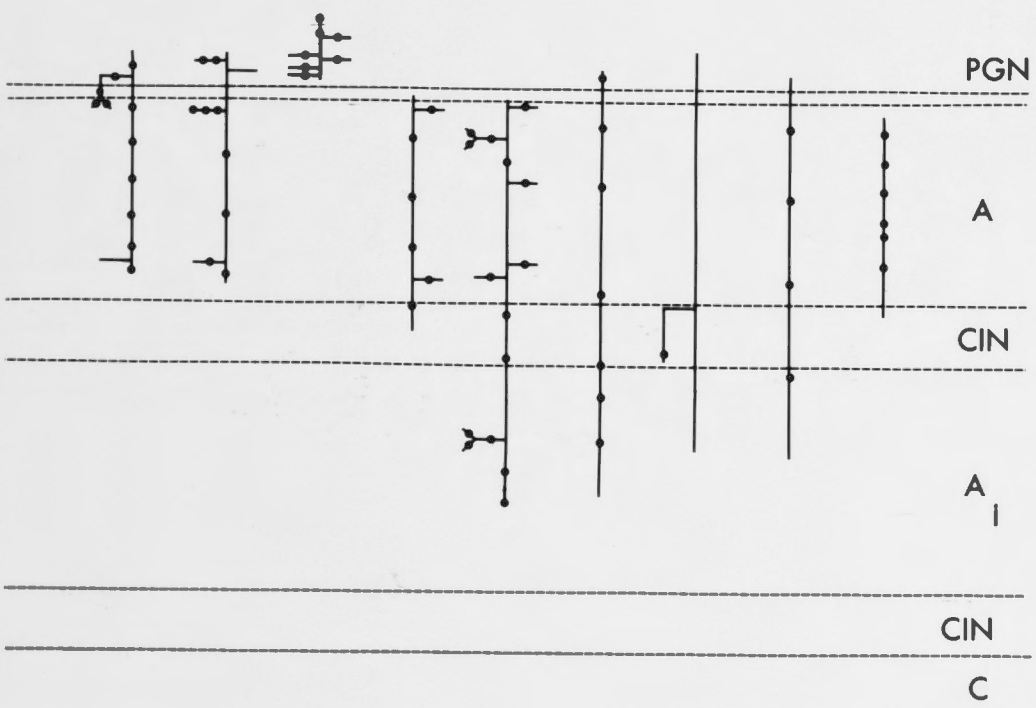
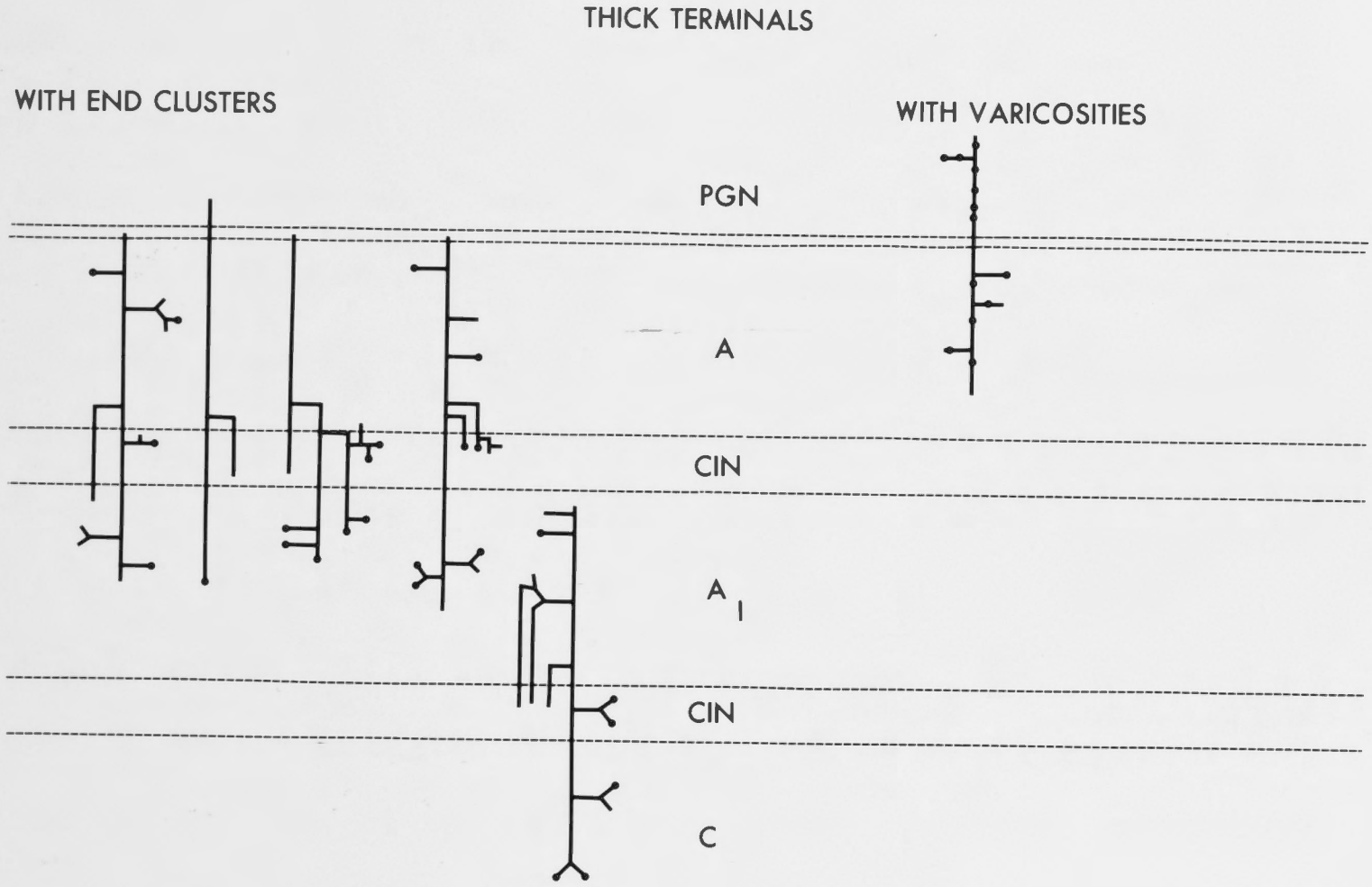


Fig. 3: Schematic repesentation similar to that of Fig. 2, giving a relative indication of the incidence of terminal swellings in thick axons.


THICK TERMINALS
WITH END CLUSTERS
WITH VARICOSITIES
PGN
A
CIN
A 1
CIN
C

the resolving level of the light microscope. These fine axons however, were readily distinguished from axons larger than 1.0µm and this was chosen as the dividing point between "thin" and "thick" axons.

The branching and terminal organization of the HRP filled axons were similar to the Golgi pictures described by Guillery (1966). In the LGN, the branching of the corticofugal fibres was extremely restricted; side branches, if present at all (see Fig. 1), were few in number and travelled short distances. Swellings on the terminals, which seemed to mark the existence of a synaptic contact, were present either as clusters at the ends of short spines, or as varicosities along the length of terminal twigs (Fig. 1 (v)). One example [Fig. 1. (vi)] appeared to terminate with a mixed arrangement of varicosities and clusters. Since many terminal twigs and boutons were observed to be filled with tracer it was concluded that labelling encompassed most of the terminal arbor.

Figure 1 shows examples of the terminal arbors of six descending axons that entered the LGN from above. The examples in Fig. 1 (i) and (ii) arose from thick axons (greater than 1µm). Despite the thickness of their main shaft these axons gave off fine terminal twigs, which carried clusters of spine-like projections with end-swelling. Generally, the thick axons had more terminal branches than the thin ones (cf. Figs. 2 and 3). Terminal clusters from thick axons were found in all layers

of the LGN but not in the PGN. On the other hand, only one thick axon was observed with varicosities along its terminal twigs and this was confined to the PGN and layer A of the LGN. Terminal clusters from thick axons appeared commonly, but not exclusively, in CIN (Fig. 3). Many individual thick axons sent terminals to at least two LGN layers as well as to CIN. When compared to the thick axons, the terminals of thin axons (Fig. 1 (iii to vi) and Fig. 2) were more often confined to a single layer of the LGN or within the PGN. This held particularly for thin axons with terminal varicosities (see the example in Fig. 1(v) which is confined to the PGN). The more restricted distribution of thin axon terminals could only be regarded as a tendency, however, since there were a number of other examples where multiple terminations occurred in more than one LGN layer, in CIN and in the PGN. There were very few examples where axons passed through a layer without developing some form of terminal swelling. Thus, axons passing to layer A_1 generally sent terminals into A, although sometimes layer A was avoided and the terminals were restricted to CIN and A_1 (Fig. 2).

DISCUSSION

A number of features in the design of corticofugal terminals could have a bearing on their functional performance. The restricted distribution and limited number of accompanying boutons or swellings suggests that each contributing cortical cell may have a limited influence on the activity of the LGN neuron. By contrast, the terminal arbors of optic tract axons in the LGN (Bowling and Michael, 1980) and optic radiation axons in the cortex (Ferster and LeVay, 1978) are much more highly ramified. The multiplicity of terminal contacts in the optic tract arborizations appear to raise the effectiveness of signal transmission since a one-for-one spike reproduction has been observed at this point (Cleland et al., 1971). On the basis of arbor size, therefore, the potential for signal relay is lower in the corticofugal pathway. In addition, transmission may also be limited by the fact that the branches of a single corticofugal terminal are not concentrated on a single LGN neuron, the majority cross layers in the LGN and presumably end on different cells.

Another feature, with functional implications, is to be seen in the higher proportion of fine axons (less than 1um at the dorsal border of the LGN) in the corticofugal than the afferent pathway (cf. Ferster and LeVay, 1978). As a result, signal conduction in the majority of corticofugal axons is comparatively slow and Harvey (1980) recorded a range of conduction times from 2ms to 29ms (mean: 6.5ms)

(see Chapter 3) in efferent S cells from lamina 6 of the striate cortex. By contrast, signal passage through the afferent pathway to the cortex is of such short duration, occuring in less than 1ms in the axons of X and Y cells, that it is often difficult to obtain consistent measurements (Bullier and Henry, 1979a).

With regard to the distribution of axons of different diameter in the corticofugal pathway my findings fail to confirm the proposal that thick axons terminate exclusively in the PGN (Harvey, 1978) or in CIN (Guillery, 1967). It follows if C and S cells have thick and thin axons, respectively (Harvey, 1978), then each type must project to a variety of locations throughout the LGN and PGN (see chapter 3). The results in Chapter 3, however, show that the segregation of conduction velocities in the axons of S and C cells is not complete and that the termination of thick and thin axons may not be as exclusive as previously believed.

For both S and C cells in the corticofugal pathway, however, the morphology favours an arrangement in which serial contacts are made at different points in the LGN and PGN. When these multiple contacts are made across layers in the LGN, this sequential termination could serve as a basis for binocular interaction; when made between the PGN and the LGN, the link could exert a co-cordinating influence on inhibition passing between the two nuclei (Dubin and Cleland, 1977; Friedlander et al., 1981). Whatever its role, the serial link between the PGN and LGN

seems to rule out the exclusiveness of the C and S cell termination proposed by Harvey (1978).

CHAPTER 3

PHYSIOLOGICAL PROPERTIES OF PGN AND LGN CELLS RECEIVING FROM LAMINA 6 CELLS OF THE STRIATE CORTEX

INTRODUCTION

In the past, the physiology of the corticofugal pathway and its contributing and recipient cells has been investigated in two ways. The first, involves the study of cortical cells driven antidromically by electrical stimulation in the LGN or PGN (Harvey, 1978). The second, examines trans-synaptic responses in LGN and PGN cells following electrical stimulation in the cortex (Cleland et al., 1976; Dubin and Cleland, 1977). As mentioned in Chapter 2, Harvey proposed from studies of this type that the faster conducting axons, arising from C cells, terminated in the PGN while the slower conducting axons of S cells terminated in the LGN. The distribution of terminals from large and small axons described in Chapter 2 failed to support Harvey's hypothesis. The present study, seeks to explain this inconsistency by looking once again at the trans-synaptic conduction times for signals travelling from the striate cortex to cells in the PGN and LGN Cleland et al., 1976; Dubin and Cleland, 1977). New measurements were also made of antidromic conduction time in the axons of striate neurons activated by a stimulating electrode in the LGN (Harvey, 1978).

The AIM of the present study

The two earlier experiments measuring axonal conduction times in the corticofugal pathway to the LGN (Dubin and Cleland, 1977; Harvey, 1978) were carried out in different laboratories and produced rather small samples of cells. The present study was undertaken to increase the sample size and to allow a direct comparison of conduction times for both directions of signal passage in the corticofugal pathway. At the same time, the response properties of PGN cells, and to a lesser extent LGN cells, were examined to see if they reflected any of the response characteristics of either the S or C cells of the striate cortex.

METHODS

(Note: The methods presented in this chapter apply in for all the physiological experiments in later chapters)

Ten cats were used in the study. Each was about one year old and weighing between 2.Ø and 3.5kg. During surgery the animals were anaesthetized with halothane (induced with 4% and maintained at 1.5%) mixed with N_2O:O_2 (7Ø:3Ø). After surgery and during recording, the anaesthetic was N_2O:O_2 (7Ø:3Ø) supplemented with Nembutal (1mg kg^{-1} h^{-1}) (Hammond,1978). Local anaesthetic (Marcain:Astra) was injected around all wound margins.

A cannula with an inner sleeve was inserted in the trachea. The sleeve could be withdrawn to remove the mucous that had accumulated at the base of the cannula. Eye movements were reduced by paralysis of the extra ocular muscles and by bilateral cervical sympathectomy. The paralysis was achieved with a continuous infusion of pancuronium bromide (Pavulon:Ø.4mg kg^{-1} h^{-1}) and gallamine triethiodide (Flaxedil; 5mg kg^{-1} h^{-1} in depolyte (Intervet (Australia Pty. Ltd.). The paralysed animal was artificially respired with the N_2O:O_2 mixture delivered at a rate of 2Ø-25 strokes per minute. Stroke volume varied from about 4Ø to 5Øml per stroke to maintain the expired end-tidal CO_2 level at 3.5% to 4%. The animal's temperature was maintained with the aid of a thermostatically controlled electric heating blanket. Heart rate and ECG were monitored continuously. An antibiotic, triplopen (Glaxovet), was injected

intramuscularly (1ml) into the cat and occasionally 2.5mg Hydrocortisyl (Roullel) was similarly administered.

The corneas were protected with plastic contact lenses of zero power and the eye lids and nictitating membranes were retracted after the sympathectomy by instilling 10% neosynephrin. The pupils were dilated with 1% atropine. To counter possible infection, Neosporine (Wellcome laboratories) eye drops were installed into each eye. A Zeiss fundus camera was used for projecting retinal landmarks and for centering 3mm diameter artificial pupils. The blind spot and area centralis of each eye were plotted at intervals during the course of the experiment in order to monitor eye position. The animal was refracted using a retinoscope and correcting lenses were fitted accordingly. During surgery, small craniotomies were performed for the insertion of the recording and stimulating electrodes.

For recording, single units were isolated extracellularly with a tungsten-in -glass microelectrode (Levick, 1972) (exposed tip around 8µm in length) which was advanced in steps of 1um with a Kopf microdrive. The recording electrode was driven down until contact was made with the cortex. This was recognized by a sudden increase in the background noise level followed usually within a distance of 100-200µm by a cell death. Thereafter, the electrode was advanced at 1µm steps using an electrically driven Kopf microdrive. Conventional differential amplification and filtering (band pass:60Hz to 6000Hz) were used to record the activity of single neurons isolated by

the eletrode. Extracellularly recorded action potentials were monitored by listening to audioamplifier and watching a cathode ray oscilloscope. The recording electrode was positioned in different locations for the two series of experiments described in this chapter. Firstly, it was placed in the thalamus close to HC AP 6.Ø, ML 9.Ø at the depth of 12-15mm; and then for the second experiments in the striate cortex near HC AP -3.Ø, ML 1.Ø.

The stereotaxic head holder used in these experiments was similar to that described by Bishop et al., (1962a). The animals head but not its body, was tilted forward 12.5 degrees to the horizontal so as to bring the visual axis into the horizontal plane and perpendicular to the tangent screen. The general arrangement of the cat and the hand plotting table was similar to that used previously (Bishop et al., 1971). All receptive fields were plotted using hand held visual stimuli. Slits or spots of light were projected on the plotting table by means of a hand held Keeler projectoscope and black targets were made from thin cardboard. The receptive fields were plotted on sheet of paper and kept as a permanent record of the location and general properties of each isolated unit.

Stimulating electrodes were constructed from tungsten wire insulated with glass. The exposed tip protruded about 1mm from the end of the insulation. Bipolar stimulating electrodes were always used and current was passed between pairs of electrodes.

The criteria used to distinguish antidromic from

orthodromic activation were based on those of Bishop et al., (1962b). Although the main test was the "collision test" (Fuller and Schlag, 1976). Collision can be defined as the situation in which two impulses travel towards each other along a nerve fibre, collide and cancel each other. In antidromic firing, the cell's own axon is stimulated electrically at the stimulating site and the action potential travels back along the axon to the cell body. If a natural action potential is generated at the cell body and travels in the opposite direction down the axon there is the possibility of collision. An action potential initiated at the stimulating site will not be recorded at the cell body if it occurs just after the natural action potential. Should the natural or spontaneous spike have moved beyond the stimulating site, then the electrically activated spike will pass unimpeded to the cell body. There is a maximum time during which no response will appear at the cell body; this equals the time to travel from stimulating site plus a refractory period for the axon fibre.

Tests, other than that for collision, were used to confirm whether there was antidromic activation. These included fractionation of the waveform at high stimulus frequencies (Bishop et al., 1962b) and the absence of latency jitter. Orthodromic action potentials do not normally follow high frequencies of stimulation and often have great variation or jitter in their response latencies to repeated stimulation. The differences in ortho- and anti-dromic firing arise because only the orthodromic

response is subject to the variations that occur when the signal crosses a synaptic junction.

In practice, stimulating currents were varied between $1Ø^{-6}$A and $1Ø^{-2}$A but in most instances thresholds were found to be around 1ØØμA for a stimulating pulse of 2ØØμs. Through out, the polarity of the stimulating pair was repeatedly reversed, usually after delivering around 1Ø shocks. In the first experiments described in this chapter, a pair of electrodes (OR_2) were placed in the primary visual cortex close to HC coordinates: AP 3.Ø; ML 1.Ø. For the sencond group of experiments the stimulating electrodes (OR_1) were positioned in LGN (HC AP 6.Ø, ML 8.Ø depth about 12 to 14) and and in the superior colliculus (SC) (HC AP 2.Ø ML 1.5 at a depth of 15mm). The final position of the OR_1 and SC electrodes were established by recording the maximum level of multiunit acitivity produced by a flashing visual stimulus. Craniotomies were covered with a protective layer of agar.

Electrolytic lesions (5μA/5sec, tip negative) were made at selected points during the recording penetration. After the experiment, the animal was intravenously injected with Nembutal (Abbot laboratories), and perfused through the heart with 1Ø% formal saline. Nissl stained frozen sections (6Øμm) were then prepared to reconstruct the electrode tracks. It was possible to estimate the shrinkage factor for cortical tissue by measuring the distance between lesions and comparing this with the distance travelled on the Kopf microdrive while recording

was in progress. The positions of the stimulating electrodes were also checked either by gross dissection or histologically by sections prepared in a way similar to that used for identifying the locations of the recording electrodes.

A cathode ray oscilloscope under computer control was used for generation of visual stimuli for the quantitative measurement of the response patterns of recorded neurons (Bullier et al., 1982, Henry et al., 1983, Mustari et al., 1982). A raster created with a time base of 1ØØHz (1Øms/sweep) and a scan of 5ØKHz in the cathode-ray oscilloscope, produced an image of a 1,ØØØ parallel lines across the screen. Each of these lines could be resolved electronically into 256 divisions. Light and dark bars were constructed under computer control by intensifying or dimming. The area of screen covered by the raster could be varied but was set to be either 9 x 9 degrees or 18 x 18 degrees. With 9 x 9 degree area, the smallest possible bar was Ø.5' arc wide and 2.Ø' arc long. Changes in bar orientation were achieved by rotating the raster, while movement of the bars came by displacing the points of intensifying or dimming to a new line with each generation of the raster (every 1Øms). Experiments using different velocities of movement were performed in two degree/sec steps. The multiple histogram method (Henry et al., 1973) was used for each test. The histograms were stored in a computer for further analysis.

RESULTS

The emphasis of this series of experiments was directed principally towards latency measurements that reflected conduction times in the corticofugal pathway passing from the striate cortex to the LGN. Times were recorded for signal conduction in both directions in the corticofugal pathway so that, in the first experiments, the recording electrode was in the PGN on the LGN and the stimulating electrode in the striate cortex. In the second experiments, the recording electrode was in the striate cortex and stimulation was applied at the LGN. For the first group of experiments, visual response characteristics were also recorded for trans-synaptically driven cells in the PGN and the LGN.

VISUAL REPONSE PROPERTIES

PGN Cells

The visually responsive cells of the PGN were encountered within a zone that was Ø.5mm thick above the first recorded relay cell in layer A of the LGN. This region is generally identified as belonging to the PGN (Sanderson, 1971). In agreement with Sanderson, it was found that the first cells recorded in the PGN, about 1-2mm above layer A, were often difficult to stimulate visually. In contrast, cells close to the LGN usually responded to visual and cortical stimulation. These cells gave ON-OFF responses to small spot stimulation throughout their receptive field although one phase of this response, the ON

or the OFF, usually predominated. Most of these cells could be excited from both eyes but there was a variation in the strength of the response from each eye. Most cells also displayed orientation specificity and direction selectivity. The tuning curve for orientation specificity was relatively broad and was much closer to that of C than S cells of the striate cortex.

PGN units responded well to rapid movement of large white and black targets. They had receptive fields that measured about $5^{o}x5^{o}$ although a lack of consistency in their visual responses often made it difficult to draw the boundaries of the receptive field. Difficulties in detecting a response also arose from the cell's high spontaneous activity and the tendency of the response to persist after removal of the stimulus. Once the LGN was reached with the electrode the cells became clearly responsive to visual stimuli.

LGN Cells

The second group of trans-synaptically activated neurons were found in the LGN. All cells had concentrically organized receptive fields and were entirely different from ON/OFF units of the PGN. These cells were typical LGN units with concentric receptive fields with either ON or OFF centres and sustained or transient responses. No sign could be detected of the orientation or direction dependence that characterized the responses of the striate neurons contributing to the corticofugal pathway.

CONDUCTION PROPERTIES

(i)Thalamic cells; stimulating electrode in striate cortex:

PGN Cells

Trans-synaptic latencies for electrical stimulation in the striate cortex (OR_2) were recorded for 26 PGN cells. The sample distribution for these latencies presented in Fig 1A, shows that a majority (15/26) had latencies less than 2.Øms while around a third (9/26) had latencies greater than 2.Øms and in only 3 cells was the latency greater than 2.5ms. Nine cells displayed multiple spiking in response to a single electrical shock and the possibility exists that indirect pathways pass through one PGN cell to another (cf. Bullier and Henry, 1979a).

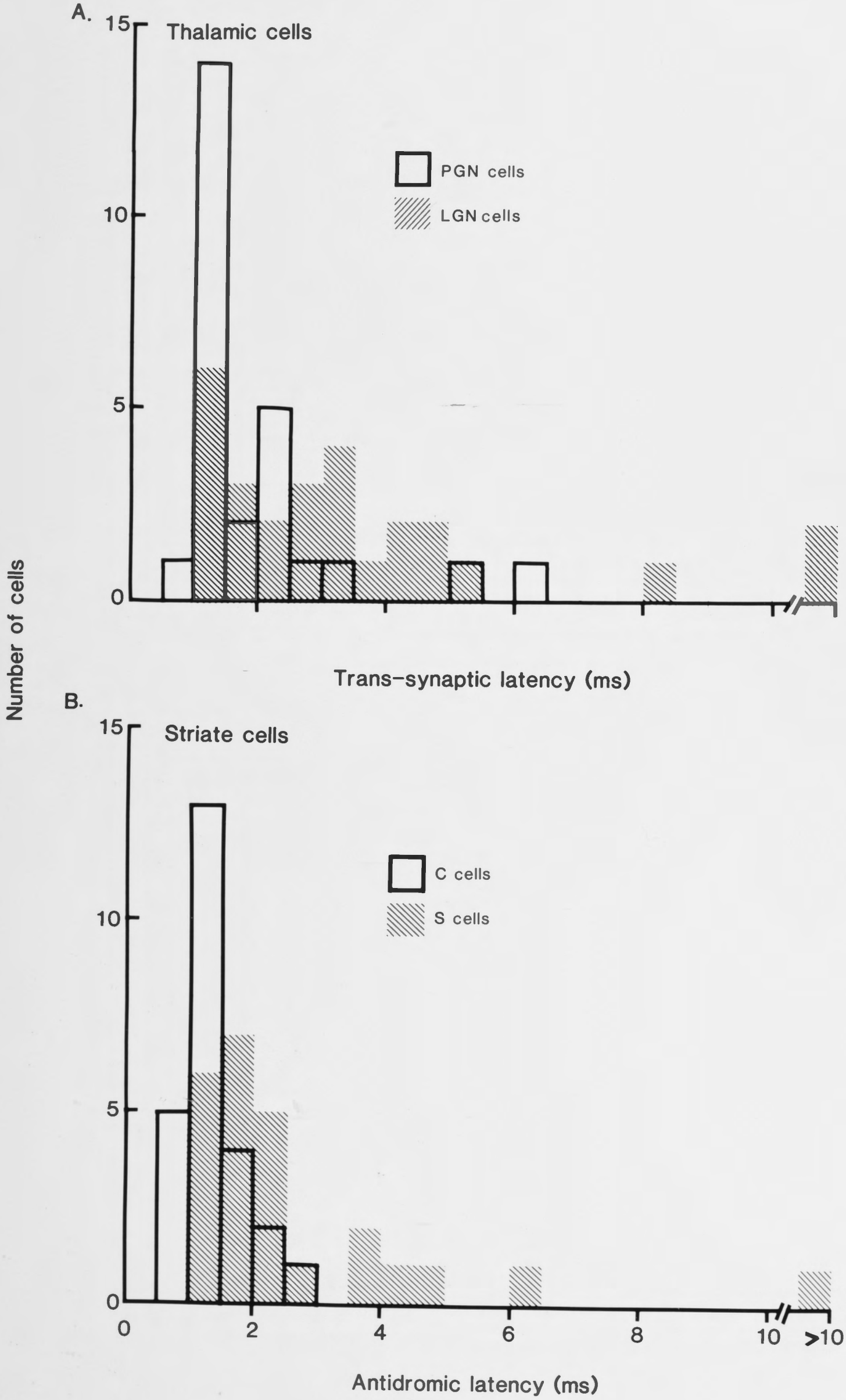
In these experiments with cortical stimulation a small sample of four PGN cells was antidromically driven from the striate cortex. The existence of such a group demonstrates that PGN cells project to the striate cortex. That these were cells and not fibres of passage was shown by the fact that two of the four cells could also be driven trans-synaptically from the striate cortex.

LGN Cells

As a group, the 27 LGN cells with a trans-synaptic drive from the cortex, tended to have longer latencies than PGN cells. Thus, as Fig. 1A shows, the majority (21/27) of cells had latencies greater than 2.Øms and that a half of the sample had latencies longer than 2.5ms.

Fig. 1: A. The distribution of trans-synaptic latencies recorded from the responses of cells of perigeniculate nucleus (PGN) and lateral geniculate nucleus (LGN) to cortical stimulation. B. Distribution of antidromic latencies recorded from the responses of S and C striate cells to thalamic stimulation.

RESPONSE LATENCY



As in the recording of PGN cells, 13 LGN cells were also driven antidromically from the cortex. The cells had short latencies typical of the geniculo-striate pathway and 9 of the 13 had latencies of less than 1.5ms.

(ii) striate neurons; stimulating electrode in the LGN

For electrical stimulation in the LGN (OR_1) antidromic drive was recorded in 26 S cells and 26 C cells. The response latencies of these cells in the distribution histograms in Fig 1B, show that there was a tendency for C cells to have faster conducting axons than S cells. Thus, 17 of the 26 C cells had latencies less than 1.5ms. While 20 of the 26 S cells had latencies greater than 1.5ms.

A comparison of the paired histograms in Fig. 1A with those in Fig 1B, reveals a close correspondence in the distributions of latencies in the different subgroups -C cells with trans-synaptic axons to the PGN and S cells with trans-synaptic axons going to the LGN.

It is possible, in stimulating at OR_1 in the LGN, to activate fibres of passage on their way to the superior colliculus. To restrict the number of cells of this type, all cells with an SC drive were excluded from the sample. The likelihood that the axons were destined for the LGN was also enhanced by the fact that half the population of neurons (26) had confirmed locations in lamina 6. Only 2 cells occurred in lamina 5 and laminar locations were not ascertained in the remainder.

DISCUSSION

The central question to emerge from both the anatomical and physiological studies on the corticofugal projection from lamina 6 is whether there are distinctive pathways terminating in the PGN and the LGN. Harvey's (1978) proposal that C cells, with their larger and faster conducting axons, ended in the PGN while S cells projected in the LGN, seems to lack support from the measurements recorded for axon sizes in Chapter 2.

The results of the present series of physiological experiments, suggest that the earlier studies may have promoted a misleading view of the level of segregation in latency measurements of subgroups based on the C and S cells in the cortex (Harvey, 1978) and their links with the PGN and the LGN, respectively (Dubin and Cleland, 1977). As is apparent in Fig. 1, the measurements from these subgroups shows some degree of overlap so that the C cell to PGN and the S cell to LGN dichotomy is not so clearly expressed in latency measurements nor, therefore, in axonal thickness, as was orginally anitcipated. In the terms of Harvey's hypothesis, therefore, it is not so disturbing, in the experiments of Chapter 2, to find a mixture of thick and thin axons passing to both the PGN and LGN. Providing most of the thick axons in the corticofugal pathway go to PGN and the thin to the LGN, then there is no inconsistency with the latency measurements of the present experiments. One feature of the anatomical experiments still at odds with Harvey's hypothesis, however, is the observation in

Chapter 2 that some corticofugal axons send terminals to both the PGN and LGN. Because of this double innervation, the hypothesis may require qualification in the future but in the meantime supporting evidence appears to come from other sources.

The receptive fields of PGN cells, although less visually reponsive than typical C cells in the striate cortex, none the less, share many of the features of the striate neuron. In particular, receptive fields with composite ON and OFF reponses are common amongst the two groups of cells. The size of the receptive fields and the response specificity of PGN cells are also much more like those of C than S cells.

Evidence of any cortical input into the response patterns of LGN cells was not obvious and even though all phases of the response were examined thoroughly there was no sign of significant asymmetry from the concentric receptive fields of these cells. Certainly, there is no similarity between the responses of LGN cells and C cells. Until the contribution from the cortex can be identified in the responses of LGN cells, however, there will be doubt about the nature of the contributing striate neuron. The weakness of the cortical reflection in the firing of thalamic cells casts doubt on any interpretation based on receptive field properties.

The possibility that the corticofugal pathway brings inhibition to the receptive fields of PGN and LGN cells has not been pursued in the present study. The observation

that the terminals of corticofugal axons appear to make exitatory or RSD synaptic contacts (Guillery, 1966; 1967) however, may indicate that the pathway is not responsible for an inhibitory input.

CHAPTER 4

THE ORIGIN AND COURSE OF CORTICO-CLAUSTRAL PATHWAYS

INTRODUCTION

The claustrum has extensive visual connections, but it lacks a direct input from the primary afferent visual pathway and no input has been detected running directly to the claustrum from either the retina or the LGN. Instead, visual inputs come to the claustrum from various regions of the visual cortex. In general, these links appear to be reciprocal in nature.

A projection passing from the claustrum to the visual cortex was first suggested by Narkiewicz (1964) from results obtained in ablation experiments. This projection was later confirmed in autoradiographic studies involving the anterograde transport of radioactive aminoacids (Carey et al., 198Ø; Flindt and Olson, 1979) and also with the neuronal tracer, HRP (Carey et al., 1979; Kievit and Kuypers, 1975; Norita, 1977; Olson and Graybiel, 198Ø; Riche and Lenoir, 1978; Spatz, 1975). These investigators all agreed that the principal point of termination of the claustro-cortical pathway in the striate cortex is in laminae 4 and 6.

In the other direction, the existence of cortico-claustral projections were also demonstrated initially by the ablation methods (Berke, 196Ø; Hirasawa et al., 1939; Mettler, 1935; Rae, 1954; Whitlock and Nauta, 1956). Ablation of the occipital cortex resulted in degeneration of terminals in the posterior most part of the claustrum (Carman et al., 1964; Druga, 1966; 68). Later, autoradiographic tracing also confirmed the existence of

connectivity between the claustrum and the visual cortex (Jayaraman and Updyke, 1979; LeVay and Sherk, 1981a; Olson and Graybiel, 1980; Sanides and Buchholtz, 1979; Squatrito et al., 1980) but it was the HRP tracer method that provided the first information on the cellular components of the link.

With HRP, it was demonstrated in general, that, cortico-claustral projections arose from pyramidal cells in in layer 6 (in the tree shrew- Carey et al., 1980; in the cat- Levay and Sherk, 1981a; Olson and Graybiel, 1980). The results of HRP experiments also showed that in the striate cortex only 3% to 10% (LeVay and Sherk, 1981a; McCourt et al., 1985a) of the cells in lamina 6 contributed to the cortico-claustral pathway. Many of the experiments injecting tracer into the claustrum reported on the presence of a labelled cells in different parts of the visual cortex, but they provide no details on the diversity or strength of these links. The present study was devised to examine the range and anatomical arrangement of the various neural loops linking the claustrum with different parts of the visual cortex.

The AIM of the present chapter

The present study set out to discover the range of the neural connections between claustrum and various parts of the visual cortex. The tracer, WGA-HRP, was injected into the claustrum and different cortical regions were evaluated for retrogradely labelled cells and anterogradely labelled afferent terminals. The search for the transported tracer extended into both hemispheres and also led to the construction of a map of the intermediate course taken by the claustro-cortical pathways. The map of the intermediate course of the pathway was to provide guidance in the positioning of stimulating electrode for the experiments in Chapter 5.

METHODS

This study includes data from 5 cats about 12 months old and weighing 2.Ø-3.5kg. In general, the methods are is similar to those of used for Chapter 1. In brief, WGA-HRP (less than Ø.1µl of 5% tracer in phosphate buffer) was injected into the visual segment of the claustrum. The injection was given under analgesic and anaesthetic cover and the animal was allowed to survive for 48 to 72hrs. The animal was perfused under deep anaesthesia (Nembutal; 6Ømg/kg; i.v) and the histological material was prepared by reacting sections with tetramethyl benzidine (TMB) (Mesulam, 1978) (see Chapter 1).

Approach taken to inject into the claustrum

The claustrum is surrounded by the visual areas such as the anterolateral bank of the lateral suprasylvian sulcus (ALLS) (Tusa et al., 1981), which lies vertically above, and the ectosylvian visual area (EVA) (Olson and Graybiel 1981; Mucke et al., 1983), which is separated from the dorso-lateral side of the nucleus by the extreme capsule. Both ALLS and EVA have connections with other visual areas and any contamination by the tracers of these two areas, would distort the picture of claustral connectivity. If the injecting needle takes a vertical course before reaching the claustrum, then it would pass through ALLS; at an angle, it could pass through EVA or the auditory cortex. To avoid passing through these areas, a horizontal approach was taken to reach the claustrum.

In this study, a 1µl microsyringe was inserted in

different animals and, although the horizontal approach was finally adopted as the most appropriate, a variety of angles were used in the earlier experiments. The following description is based on the results of a horizontal injection but the consistency of the results with all angles of approach, suggested that there was little tracer contamination at regions outside the claustrum.

In all cases, the injecting needle entered the brain at the same antero-posterior position HC AP: +12.Ø (Reinoso-1961). The depth of the needle varied with the angle of entry but, for the horizontal line, the distance travelled was between 4 and 5mm. Histological reconstruction was used to confirm the deposition of the tracer in the visual segment of the claustrum.

Density of labelled cells

The density of labelled cells in each area was measured by making labelled cell counts in individual coronal sections that were cut in 1mm steps throughout the region of label. In each of these sections, all labelled cells were counted and then averaged over 1mm lengths of the lamina in the anterior/posterior dimension. This figure provided a more accurate estimate of the number of cells contributing to a particular pathway and was preferred to a measurement expressed in terms of unit area.

RESULTS

Examination of histological sections, after the injection of WGA-HRP into the visual segment of the claustrum and the subsequent reaction with TMB, revealed retrogradely labelled cells in as many as 14 regions in the visual cortex. In most instances, the same regions had networks of labelled terminals that may have filled as a result of anterograde transport. Below the cortex, in the white matter, it was also possible to see labelled axons and to follow their course. It was not simple to determine if this axonal filling in the white matter was the result of antero- or retro-grade transport and it will be assumed that both modes of transport could contribute to the labelled pathway.

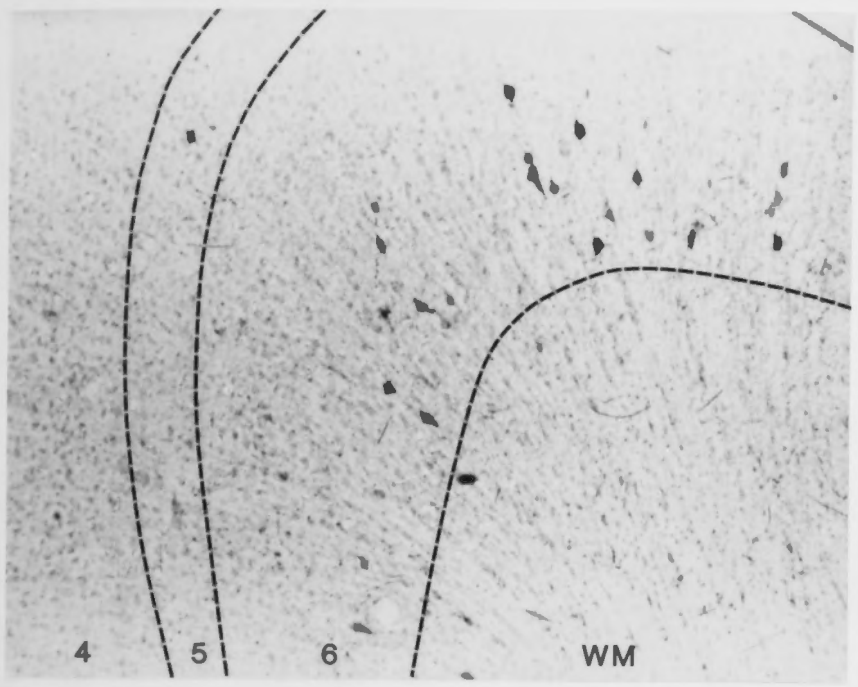
In assessing the strength of a cortical link, quantitative evaluations were made by counting from the 60 um section with the highest incidence of retrogradely filled cells. Typical distribution patterns for labelled cells are reproduced in the micrographs of different areas in Fig. 1 and the quantitative counts made from all such areas linking with the claustrum are presented in Fig. 2. Counts of this nature were conducted in cortex lying between HC A.P. -4 to +13. Outside this range, there was little evidence of retrograde filling.

Cortical links with the claustrum

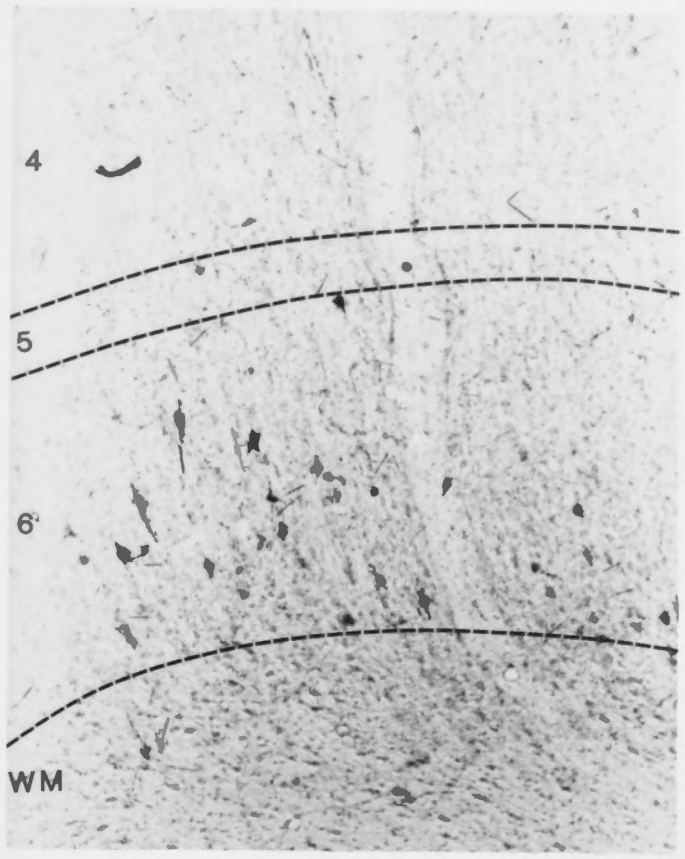
A total of 14 cortical areas of the ipsilateral hemisphere and 7 homotopic areas on the contralateral side

Fig. 1: Photo-micrographs of coronal sections showing the distribution of retrogradely labelled cells in selected cortical areas following the injection of WGA-HRP into the claustrum. Areas pictured: 1, Area 17; 2, Area 18; 3, Area 19; 4, Splenial visual area; 5, Area 20b. In 1,2,3 and 4 labelled cells projecting to claustrum are concentrated in lamina 6; in 5, labelled cells are almost equally distributed between laminae 4 and 6. Inset shows location of micrographs taken from sections at HC; AP:+8 and -4. Scale bar: 100µm.

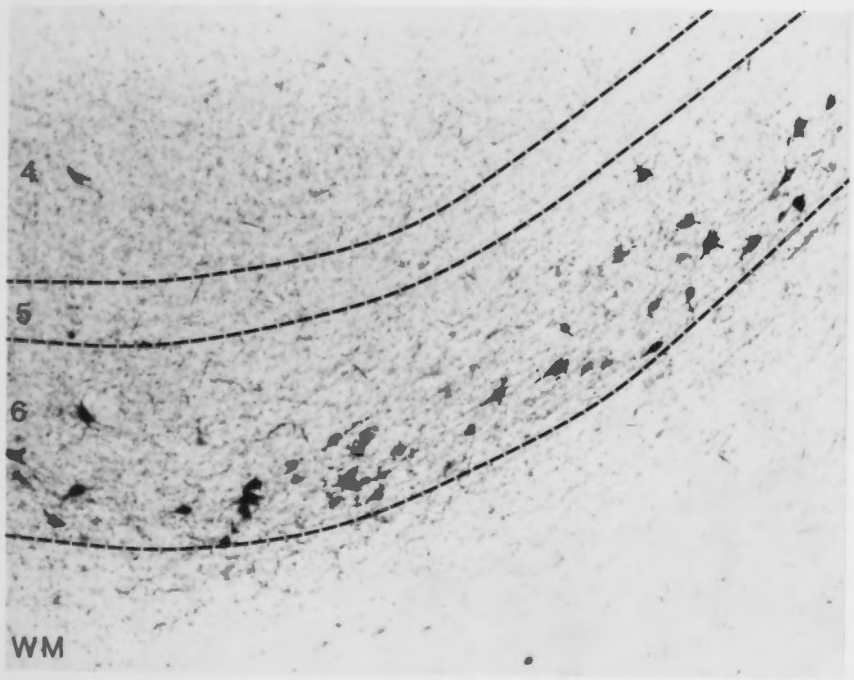
1. From Area 17



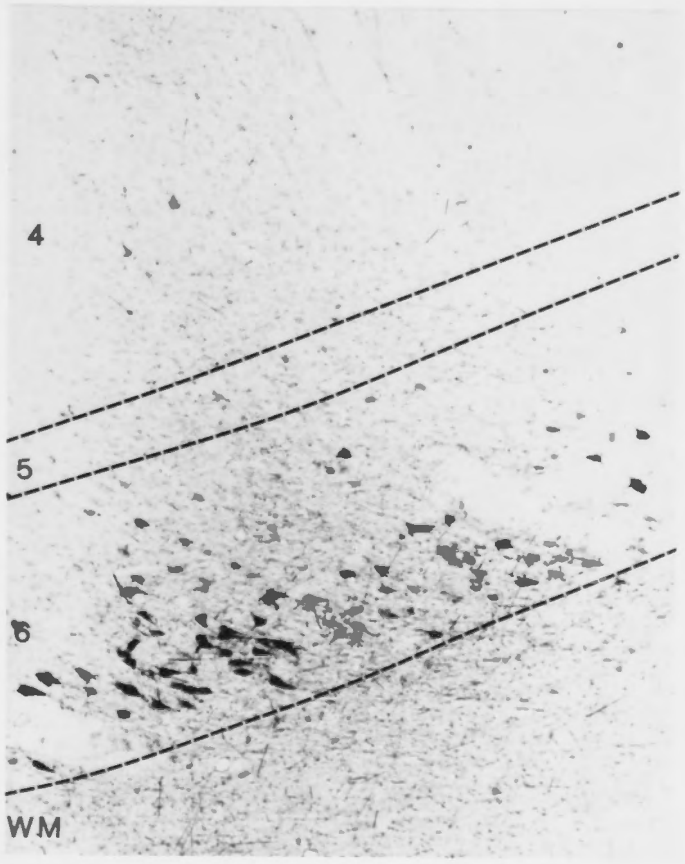
2. From Area 18



3. From Area 19



4. From SVA



5. From Area 20b

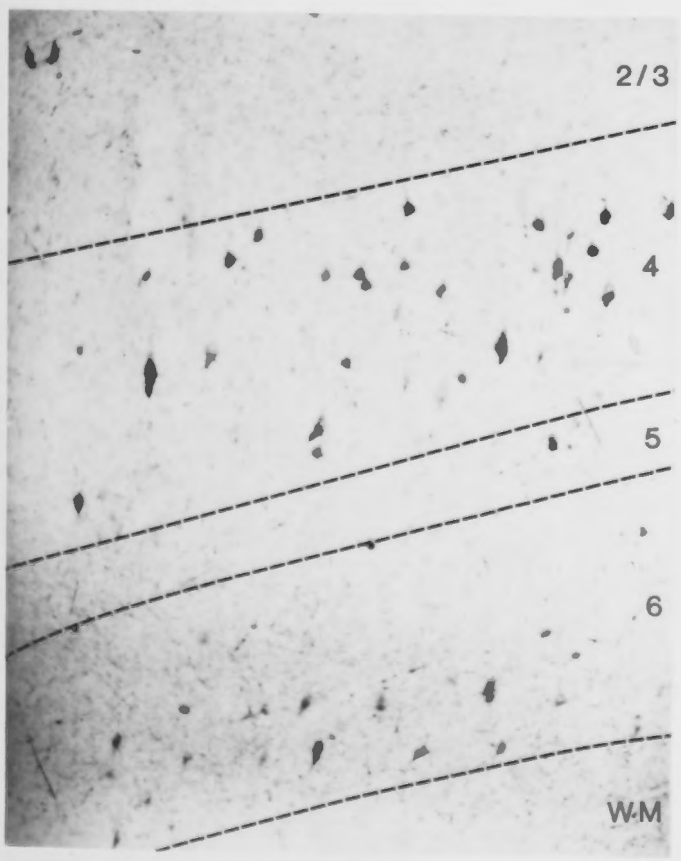
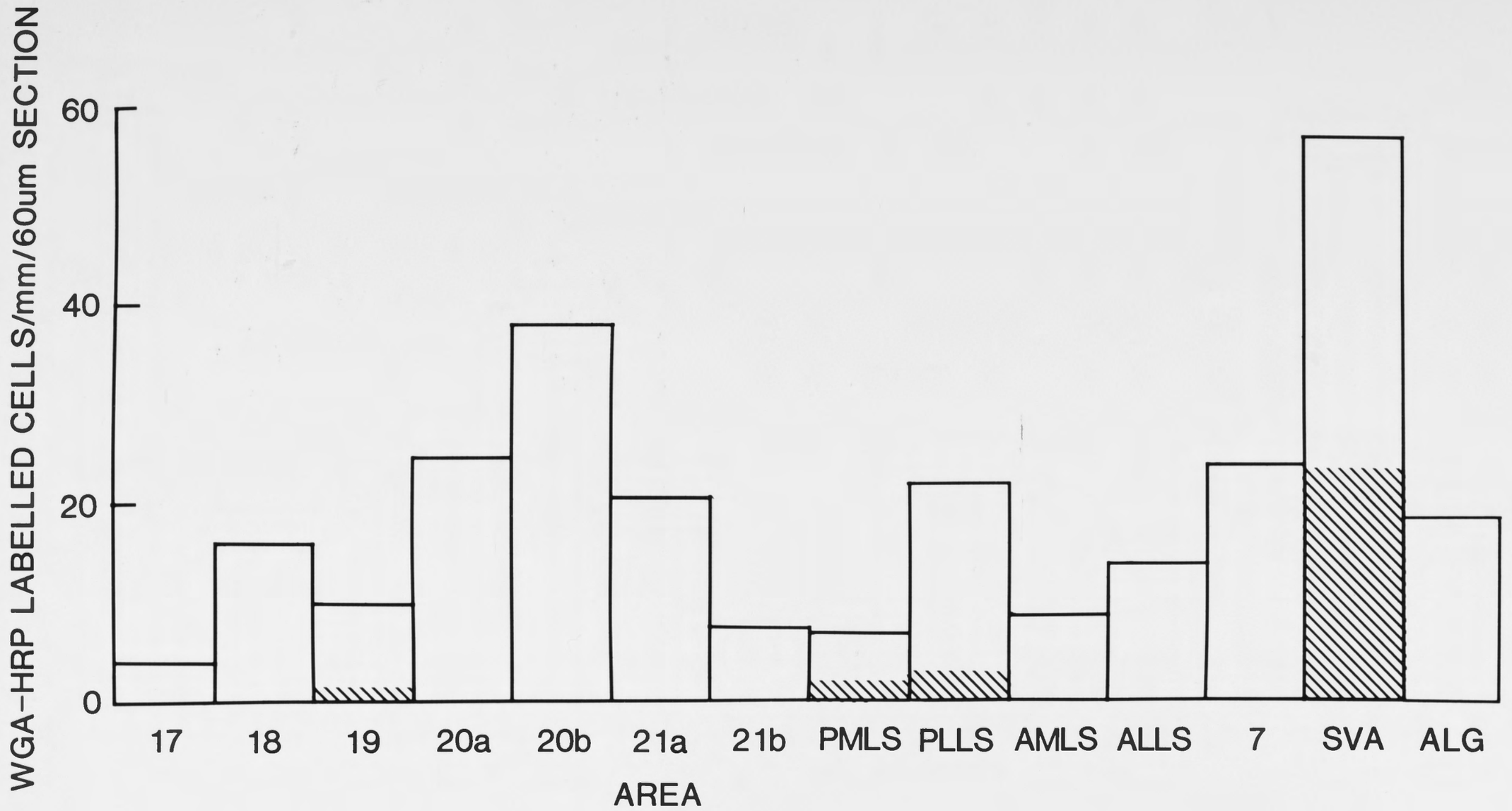


Fig. 2: Relative incidence of labelled cells in cortical areas after injection of tracer into the claustrum. Counts were averaged over 1mm wide strips in the histological section with the highest incidence. Open bars: ipsilateral cortex; hatched bars: contralateral cortex. Abbreviations: PMLS, PLLS, AMLS, ALLS: areas in the lateral suprasylvian complex; SVA: splenial visual area; ALG: anterolateral gyrus visual area.


WGA-HRP LABELLED CELLS/mm/60um SECTION
60
40
20
0
17
18
19
20a
20b
21a
21b
PMLS
PLLS
AMLS
ALLS
7
SVA
ALG
AREA

were found to contain labelled cells following the injection of WGA-HRP into the visual claustrum (see Fig. 2). All the labelled regions on the ipsilateral side also showed evidence of heavy terminal labelling. By contrast, on the contralateral side, the axon terminal labelling was much sparser or non-existent.

The results were taken from the experimental animal, in which the injecting needle was given a horizontal approach, although the findings in the other four animals were qualitatively similar. For convenience, the 14 regions containing labelled cells (Fig. 2) were grouped into 4 subgroups. The first, comprising Area 17, 18 and 19, received a direct input from the LGN. The second, contained of the 4 regions of the lateral suprasylvian complex (antero-medial, AMLS; antero-lateral, ALLS; postero-medial, PMLS; postero-lateral, PLLS). The third, consisted of Areas 2Øa and 2Øb, which had distinctive laminar distribution of retrogradely filled cells. The final group of Areas 21a, 7, ALG (anterolateral gyrus Area) and SVA (splenial visual area) was made up of the remaining areas. The term SVA is adopted with some uncertainity since it is used to describe labelling that was not only confined to the ventral lip of the splenial sulcus but also labelling that extended into the cingulate gyrus.

1. Primary visual cortex (Areas 17, 18 and 19)

Bright field micrographs in Figs 1.1, 1.2 and 1.3 were taken from a coronal section (HC AP: +8.Ø) of primary visual cortex and show the patterns of labelled cells in visual cortical Areas 17, 18 and 19 respectively. The labelled cell counts, averaged over 1 mm anterior/posterior lengths of lamina, and presented in Fig 2, show that Area 17 had fewer labelled cells than the other two areas in this group. For all animals Area 17 consistently displayed the sparsest labelling of all the labelled areas.

The laminar distribution of labelled cells was the same in the three areas. Lamina 6 had the highest count and only occasional labelled cells were found in the other laminae. Of the three areas in this group, only Area 19 (see Fig 2) showed any evidence of labelled cells in the homotopic area in the contralateral hemisphere and even then the count was only about a tenth of that recorded on the ipsilateral side.

2. The lateral suprasylvian complex (ALLS, PLLS, AMLS and PMLS)

Labelling in the lateral suprasylvian complex did not differ in kind from that observed in the primary receiving cortices and the labelled cell counts were similar to that seen in Areas 18 and 19. For areas in the suprasylvian complex, PLLS had the greatest density (Fig. 2). As with the primary receiving cortices, there was little evidence of labelling in the cells in the homotopic regions of the

contralateral hemisphere.

3. Areas 2Øa, 2Øb

As shown in Figs. 1.5 and 2 there was heavy retrograde labelling in Areas 2Øa and 2Øb of the ipsilateral hemisphere. No labelling was detected in the corresponding areas of the contralateral cortex. In contrast to the areas represented in groups 1 and 2 above, there was a distinctive laminar distribution of labelled cells in Areas 2Øa and 2Øb. The micrographs in Fig. 1 compare the distributions of labelled cells in Area 17, 18, 19 and SVA (Fig. 1.1-4) with those in Area 2Øa (Fig. 1.5). The outstanding feature in Area 2Øa was the extension of retrograde labelling into lamina 4 and a similar distinction held for Area 2Øb. Figure 3 shows that the counts of labelled cells in lamina 4 of Areas 2Øa and 2Øb were just in excess of those recorded for lamina 6.

4. Areas 21a, 21b, 7, ALG and SVA

In this miscellaneous group, all areas, with the possible exception of Area 21b, displayed a high incidence of labelled cells. This was particularly true of the SVA (see Fig. 1.4), which, in all tested animals, showed the heaviest labelling of all affected areas. As Fig. 2 shows, this high level of labelling also extended to SVA in the contralateral hemisphere, where the incidence of labelled cells was similar to that found in the heaviest labelled regions of the ipsilateral side. As in all regions, other than Areas 2Øa and 2Øb, the labelling in

this group was confined principally to lamina 6.

Laminar distribution of retrogradely labelled cells

Figure 3 shows the distribution histogram of labelled cells, found in the different cortical regions. The open bars represent counts in ipsilateral regions and the hatched, counts in the homotopic regions of the contralateral hemisphere. Figure 3 shows the regional similarity in the counts within lamina 6, although SVA (with possible involvement of the cingulate gyrus) had the highest, and Area 17, the lowest incidence of labelled cells. Only Areas 20a and 20b had a substantial number of labelled cells in lamina 4 and in total less than 10% of the cortical cells projecting to the claustrum resided outside lamina 6.

The intermediate course of claustro-cortical pathway

Following an injection of WGA-HRP into the posterior segment of the dorsal half of the claustrum it was possible to trace a clearly defined pathway of labelled axons in the white matter between the claustrum and the various linking cortical regions. Figure 4 reproduces dark field micrographs showing this stream of labelled fibres travelling through white matter at HC AP levels +6, +7 and +8. Schematic representations of these and neighbouring sections are presented in Figure 5 where the range of Horsley-Clarke planes is extended from AP +4 to +12. From Figs. 4 and 5, it is apparent that the stream of labelled axons, possibly displaying both retro- and antero-grade

Fig. 3: Laminar distribution of labelled cells in cortical areas after tracer injection into the claustrum. Open bars: ipsilateral cortex; hatched bars: contralateral cortex. Counts taken from sections with maximum incidence. Only Areas 20a and 20b have a sizable number of linking cells outside lamina 6, in lamina 4.

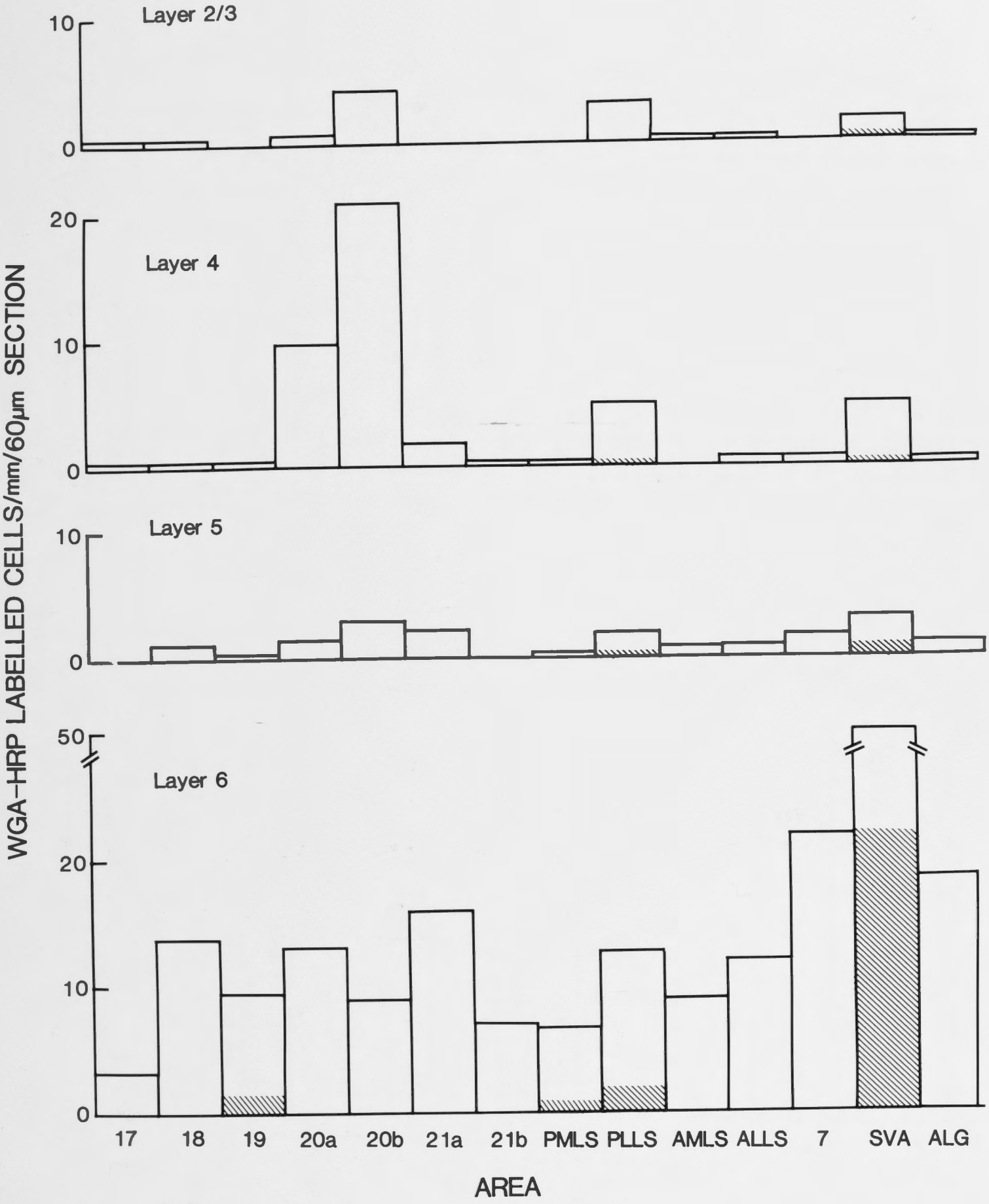
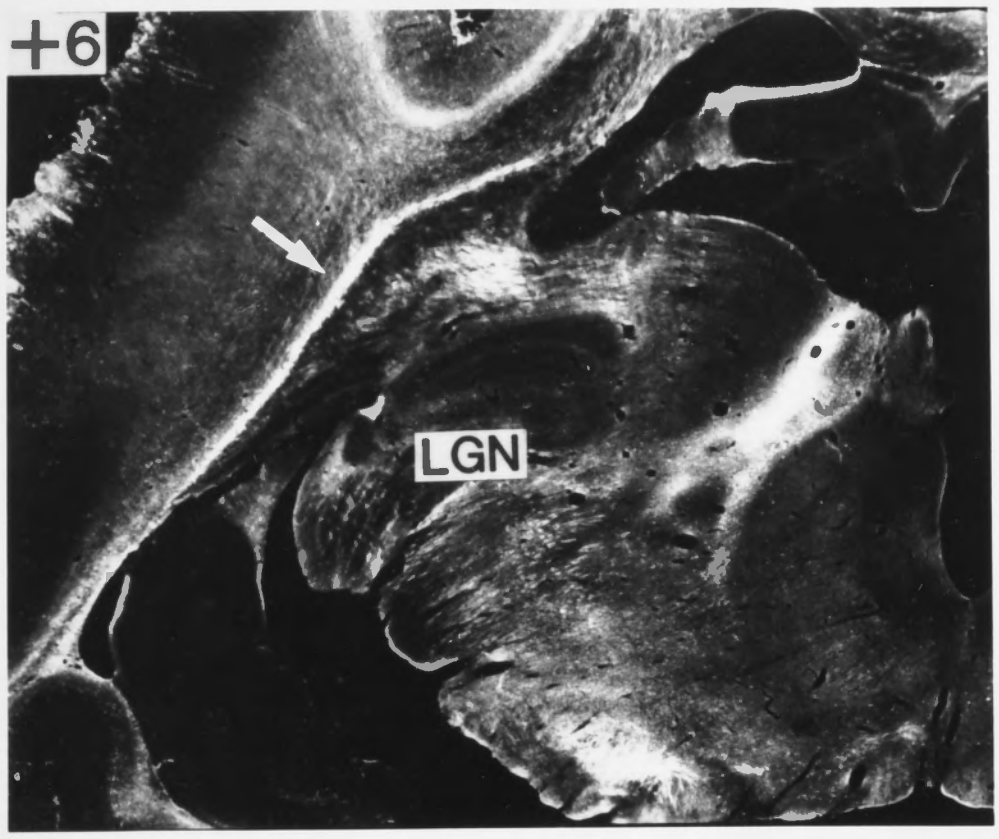

Layer 2/3
10
0
Layer 4
20
10
0
Layer 5
10
0
Layer 6
50
20
10
0
WGA-HRP LABELLED CELLS/mm/60µm SECTION
17
18
19
20a
20b
21a
21b
PMLS
PLLS
AMLS
ALLS
7
SVA
ALG
AREA

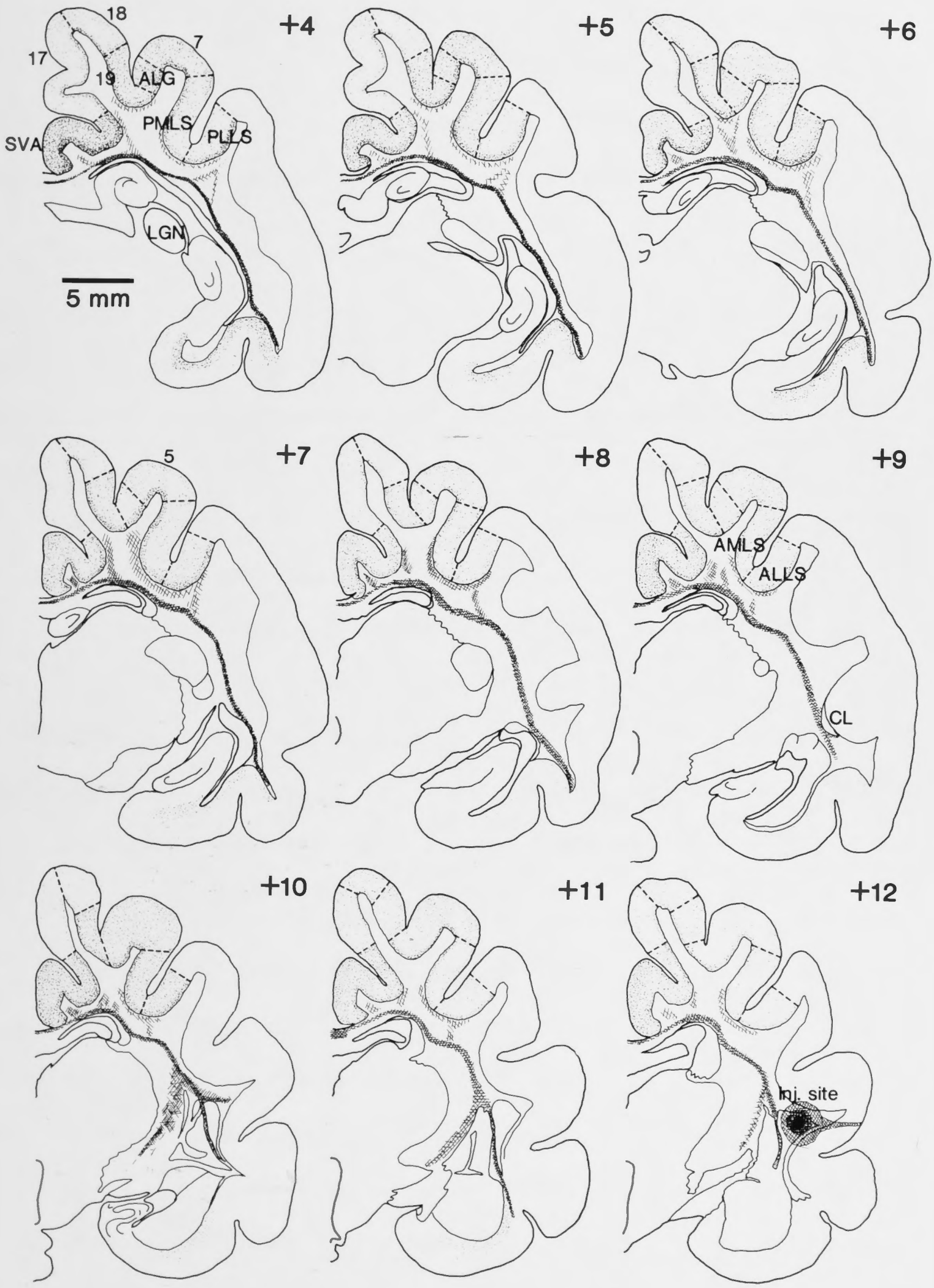
Fig. 4: Dark field photo-micrographs taken at coronal sections at HC; AP:+6, +7, and +8, showing the labelling that outlines the course of the cortico-claustral pathway (arrow) and retrograde labelling is also apparent in the nucleus lateralis medialis to the right of the LGN in the section at HC; AP:+7.


+6
LGN


+7


+8

Fig. 5: A series of schematic pictures drawn from sections at HC; AP +4 to +12, showing the tracer injection site in the claustrum, the course of pathways linking the claustrum with ipsi and contra-lateral cortex and with the thalamus.


+4
18
17
7
19
ALG
PMLS
PLLS
SVA
LGN
5 mm
+5
+6
+7
5
+8
+9
AMLS
ALLS
CL
+10
+11
+12
Inj. site

transport, emerged from the dorsal surface of the claustrum to form a flat ribbon, Ø.25mm thick and as much as 1cm wide in the anterio-posterior plane. This ribbon of axons passed medially under the cortex of the lower lip of the ectosylvian sulcus and then curved upwards.

Continuing as a coherent band, the projection passed vertically into the corona radiata until level with the deepest layer on the upper lip of the ectosylvian cortex where it angled towards the midline at approximately 45°. This line was maintained until the fibres were level with the upper margin of the lateral ventricle when further flattening brought the path to an angle of 1Ø° to 2Ø° from the horizontal. Upto the point of the second flattening, the projection maintained an almost constant anterior-posterior relationship but, once above the lateral ventricle, it fanned out and passed caudally into the centrum semiovale where the fibres mingled with the optic radiations passing from the LGN to the cortex.

Throughout their course, the labelled axons were well removed from the LGN. The anterior tip of the LGN is only 1 to 2 mms more caudal than the tail of the claustrum but the two nuclei are separated laterally by 3 to 4 mms and, as Fig. 5 shows, the labelled axons only turned medially at a point 3 to 4mms above the LGN.

Two branches of labelled axons were observed to leave the main pathway in its progression between the claustrum and the cortex. The first, and most obvious, descended at

HC AP +1Ø to pass down rostral to the LGN. At a level below the LGN, the pathway was difficult to trace but it appeared to pass caudally to be associated with labelled cells and terminals in the lateral part of the nucleus lateralis medialis (LM) and the supra geniculate nucleus (SG). This part of the LM corresponds to the region previously described as receiving afferents from deep layers of the superior colliculus (Berson and Graybiel, 1983; Dreher, 1985). The second branching occurred in the centrum semiovale where labelled axons left the main stream, entered the corpus callosum, and passed to the contralateral hemisphere. This crossed pathway was apparent in sections that ranged from HC AP +4 to +12 (see Fig. 5).

Terminal labelling

Terminal labelling, which may have included collaterals from efferents leaving the cortical area, was observed across all laminae in all areas with retrogradely labelled cells. Under low power microscopic examination slightly denser bands of tracer were noted at the laminae 3/4 border (the upper 1/4 of lamina 4 to mid lamina 3) and in lamina 6 (cf. LeVay and Sherk, 1981b).

DISCUSSION

Ipsilateral cortical links with the claustrum

It is now well established that the claustrum makes a wide variety of reciprocal connections with different regions in the ipsilateral visual cortex. LeVay and Sherk (1981b) described some of these links but refrained from a comprehensive description on the grounds that regions within the visual cortex could not be accurately identified in histological sections. Taking the alternative approach, it appears acceptable to apply the anatomical maps prepared in recent years (Tusa et al., 1981). Some of present findings are in accord with those of LeVay and Sherk (1981b) and have confirmed that the claustrum is linked with Area 17, 18, 19, 21a and PMLS. In addition, the present results suggest that the claustrum is also linked with Areas 20a, 20b, 21b, 7, PLLS, ALLS, ALMS, ALG and SVA. Some of these supplementary areas also appeared to have carried label in LeVay and Sherk's (1981b) study and are either mentioned in passing or included, without comment, as labelled regions in their diagrams.

Possibly, a greater problem than designating the cortical destination of a tracer is the question of whether the injection is restricted to the confines of the nucleus. In all of the experiments in this series the densest part of the tracer stain was confined to the claustrum but the surrounding halo often spread into the neighbouring white matter. The prospect that the tracer in the halo may have been taken up by fibres of passage associated with other

visual areas prompted a study (McCourt et al., 1985b) to characterize any exclusive features in EVA's connectivity. When compared with the claustrum, it appears that EVA alone has links with DLS and the absence of DLS labelling in all of the claustral injections suggested that the spread of tracer outside the claustrum did not involve EVA. With similar logic the absence of label in the medial geniculate nucleus in all the experimental animals seemed to rule out the unintentional deposition of significant levels of tracer in either auditory or insular cortex (Guldin and Markowitsch, 1984).

Concerning the density of cortical linkage with the claustrum, it is significant that dense connections do not exist with Area 17, the area that receives the richest direct input from the LGN. Here, cells filled retrogradely from the claustrum were found in fewer numbers than any other labelled cortical area. The region of densest labelling was SVA, a region for which there is little information in the visual responses of its cells or on its position in any visual sequence (for properties of cells in the dorsal splenial sulcus see Kalia and Whiteridge, 1973).

Contralateral cortical links

Despite the observation that labelled axons crossed the corpus callosum in appreciable numbers, few regions of label were found on the contralateral cortex. Only the contralateral SVA had labelling that compared in density with that seen in the ipsilateral cortex. When this labelling is considered along with the heavy labelling in

its ipsilateral counterpart, the SVA appears to have a strong association with the claustrum. An interpretation of this inter-relationship must await further investigation into the functional role of the SVA and also on the separation of any contribution that may arise from the cortex of the cingulate gyrus.

Laminar distribution of cortical cells projecting to the claustrum

In agreement with LeVay and Sherk (1981b), it was found in all cortical regions projecting to the claustrum, that the cells of origin resided in lamina 6. In some regions, a contribution also came from cells in other laminae. Within lamina 6 there was a remarkable uniformity in the density of labelled cells in the various cortical areas. Only in Area 17, with a low incidence, and SVA, with a high one, did the counts appear to deviate appreciably from the overall average. For each area, the distribution of labelled cells extended across lamina 6 with the number tending to peak in the lower half of the lamina. This spread differed from the pattern found in lamina 6 cells that projected to other sites such as the LGN and other cortical regions (McCourt et al., 1985a).

The comprehensive nature of cortico-claustral linkages is apparent in the variety of contributory regions. Overall, fourteen cortical regions appear to send connecting paths to the claustrum and this diversity must cover a wide range of cortical processing. From the inputs and inter-relationships of the various areas that comprise

the visual cortex, Berson and Graybiel (1983) formulated a number of familial or regional combinations based on differences in afferent input from the thalamus (for review see Dreher, 1985). In progressing from the medial to the lateral direction across the hemisphere, the different family groups are identified from their inputs that come either from (1) the retinal receiving nuclei in the thalamus, (2) from cortical receiving nuclei in the lateral part of the lateral posterior nucleus (LPl) or (3) from tectal receiving nuclei in the medial half of the lateral posterior nucleus (LPm). Each of these families has representatives sending projections to the claustrum.

Family 1, the retinal receiving group, is represented by Area 17, 18, 19, 21a, PMLS and AMLS; Family 2, by Areas 19, 21a, PMLS and AMLS, and Family 3, by ALLS and PLLS (Dreher, 1985). The occurrence of loops linking so many cortical areas and families with the claustrum suggests that there is repeated reference to claustrally generated information. On the other hand, the apparent absence of a specific point for the injection of claustral information into the visual sequence seems to suggest that the claustrum has a general rather than selective role. It is possible, however, that the claustrum acts as an interchange area for the transmission of messages from other sensory modilities into the visual sequence. In such an arrangement, the repetition of looping through the claustrum may simply reflect a design feature in which duplication at different levels is the most effective way of integrating other senses into all stages of the visual

process. For the moment, it is presumed that interaction occurs between the different functional segments of the claustrum although intra nuclear links are yet to be confirmed.

A role as an interchange station would not exclude the possibility that the claustrum relates to a specific aspect of visual information. The finding that Area 17, the region with the most extensive representation of central retina, has the weakest link of all the visual cortical regions, raises the question as to whether the claustrum makes an insignificant contribution to acute form vision. Such an interpretation would be consistent with the observation that there is little, if any, magnification in the representation of the area centralis in the claustrum (LeVay and Sherk, 1981a). Also, as shown in Chapter 5, the trans-synaptic drive from the claustrum to Area 17, in most instances, activates C cells which receive a Y stream input from the LGN (Boyapati and Henry, 1985). A close association with peripheral vision and with the Y stream would support the argument that the claustrum makes a specific but low acuity contribution to the processing of the retinal image.

ADDENDUM TO CHAPTER 4

BILATERAL CORTICAL CONNECTIONS OF THE ECTOSYLVIAN VISUAL AREA (EVA) OF THE CAT

INTRODUCTION

Olson and Graybiel (1981) were the first to describe a "zone of exclusive visual responsiveness" near the lower lip of the anterior ectosylvian sulcus of the cat cortex. As mapped by microelectrode recording, the ectosylvian visual area (EVA) was found to extend over 20mm^2 of sulcal cortex. It was shown also that EVA had strong reciprocal links with posterior lateral bank of the lateral suprasylvian sulcus (PLLS) and Area 20 (posterior suprasylvian sulcus) and a weak link with posterior medial bank of the lateral suprasylvian sulcus (PMLS). Reciprocal connections also linked EVA with the brain stem, specifically, with the nucleus lateralis posterior (LPm) and the nucleus lateralis medialis (LM) of the thalamus. Olson and Graybiel (1981) noted that the primary visual complex (Areas 17, 18 and 19) did not project directly to EVA and further, emphasising the separation from the primary input, suggested that the cortical areas projecting to EVA also lacked an input from the primary visual areas. Recent studies (Symonds and Rosenquist, 1984a), however, have reported that both PMLS and PLLS receive inputs from Areas 17, 18 and 19.

Extracellular recordings from single cells in EVA have revealed that these cells possess large receptive fields (from 50 to 100 deg^2) (Olson and Graybiel, 1981; Mucke et al., 1982), which may extend as much as 20^o into the ipsilateral visual hemifield. In their responses, the

cells showed a preference for small visual stimuli moving rapidly in a particular direction but there was little evidence of retinotopic organization.

According to two reports (Olson and Graybiel, 1981; Mucke et al., 1982) most, if not all, of the cells in EVA may be driven by either eye and most of the cells respond more vigorously to binocular than to monocular stimuli. Despite this high incidence of binocular drive there have been few reports on the anatomy of the inter-hemispheric projections associated with EVA (Micele et al., 1984; 1985).

The AIM of the present study

This experiment was originally designed as a control measure to reveal the consequences of contamination of EVA in other experiments where tracer was injected into the claustrum. However, the present study involving the direct injection of tracer into EVA also created the opportunity for new quantitative evaluation of the ipsi- and contra-lateral connections of this newly identified visual area.

METHODS

Preparation of animal

Experiments were conducted on 1Ø cats that were approximately 12 months old and weighed between 2.Ø and 2.5 kg. The general procedure of the methods is similar to that in Chapter 1. For the injection of tracer (5% WGA-HRP) the animal was prepared with a preliminary injection and then anaesthetised with Ketamine.

For all injections into EVA, craniotomies were performed immediately lateral to the anterior ectosylvian cortex. With this opening it was possible to take an almost horizontal approach so that the microsyringe passed through little or no cortex outside EVA. The microsyringe was positioned stereotaxically in the anterior/posterior direction (HC AP = + 11.Ø to + 13.Ø) while the medio-lateral position was located visually on the lower lip of the anterior ectosylvian sulcus. Every effort was made to confine the injected tracer to the cortex in that part of the anterior ectosylvian sulcus that lay immediately above the posterior, or visual segment of the claustrum. The adoption of this site for injections into EVA followed criteria developed in earlier investigations (Olson and Graybiel, 1981; Mucke et al., 1982; Updyke, 1983). Once in position, the microsyringe was used to deliver Ø.1µl to Ø.3µl of the tracer over a period of 5 minutes. The injection was kept as small as practical to confine the tracer within the cortex of EVA and a number of injections were necessary to achieve a satisfactory

localization that did not involve the claustro-cortical pathway that skirts the anterior ectosylvian sulcus (ecsa). Diffusion was also restricted by the addition of 2% lysophosphatidylcholine (Lysolecithin, Type 1; Sigma) to the WGA-HRP.

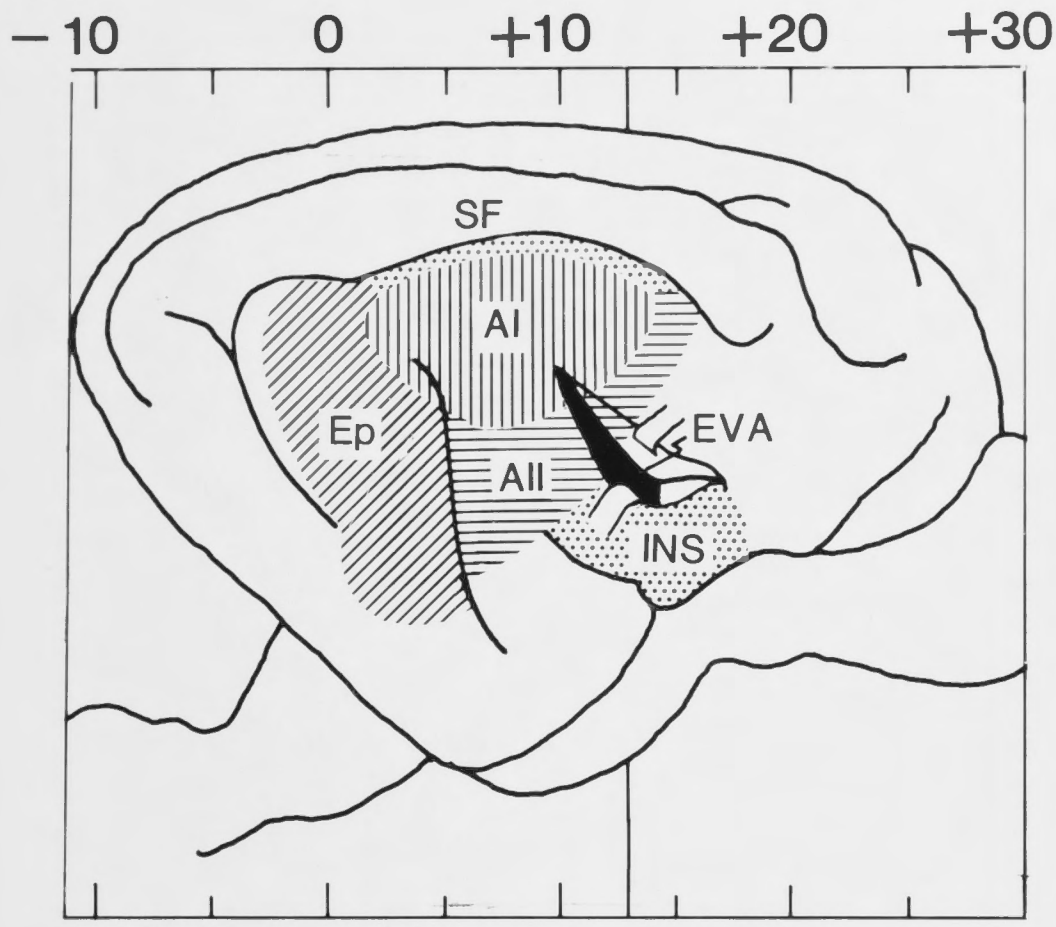
Location of EVA

The interpretation of the neural connectivity of EVA, as derived from injection of WGA-HRP, calls for a precise description of its topographical relationship to adjacent regions. In its position on the ventral lip of the ecsa, EVA (black area in retracted ecsa in Fig. 1a) lies between the auditory cortex (AII), on the upper lip of ecsa, and the insular cortex (INS). Auditory areas AI and AII of the ectosylvian gyrus lie caudal to EVA and these regions in turn lie rostral to the auditory cortex of the posterior ectosylvian sulcus (Ep). Figure 1a shows the disposition of EVA in the lateral cortex and, at the same time, provides the HC AP values (Reinoso-Suarez, 1961). These relationships are shown in more detail in Fig. 1b, which provides a cross sectional view of the brain cut along the HC plane AP+13.

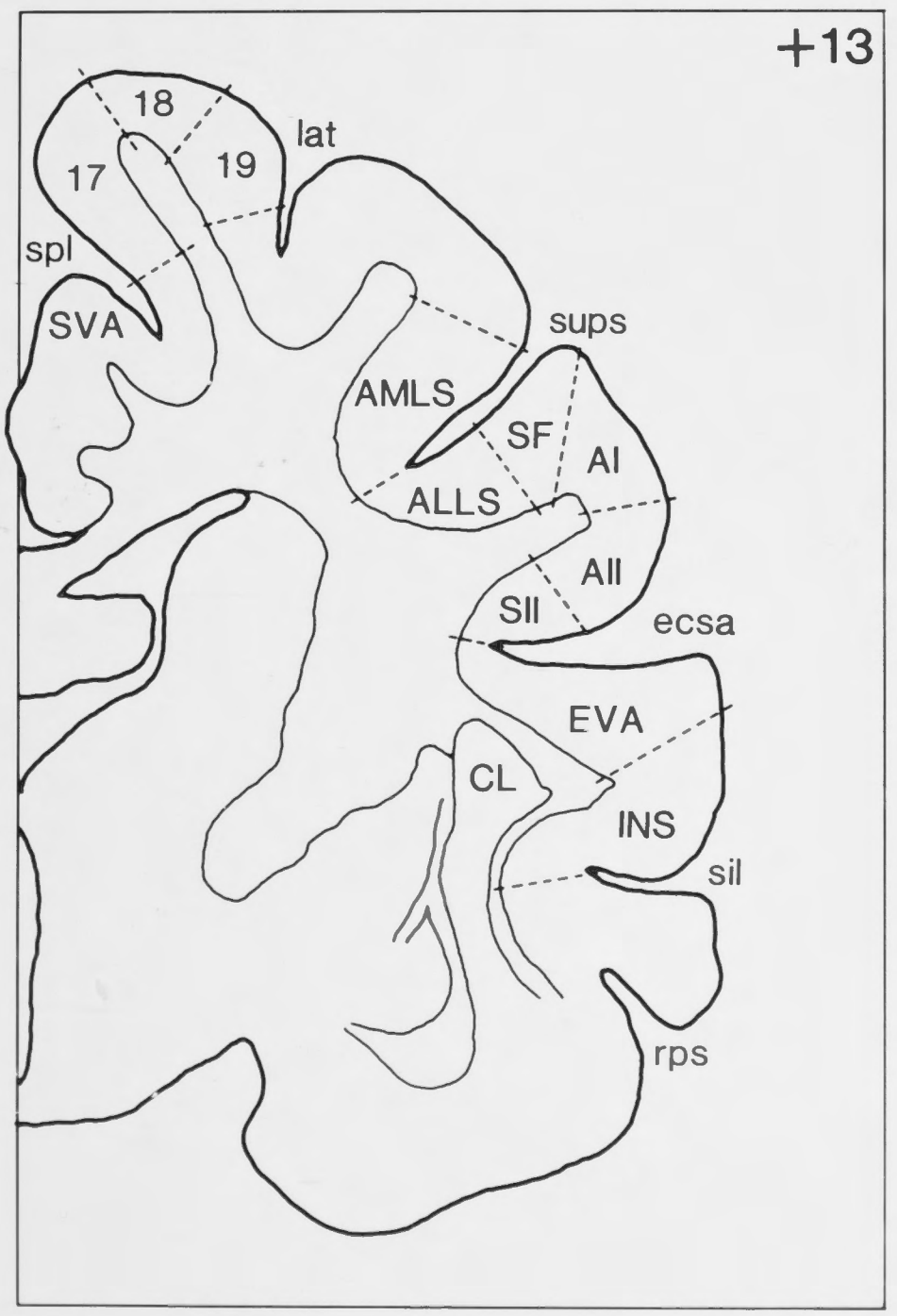
Forty eight hours after injection of the tracer, the cat was anaesthetised with Nembutal and perfused through the ascending aorta. After perfusion the brain was removed and allowed to sink in a solution of 30% sucrose for 24-48hrs before sectioning at

Fig. 1: A: Schematic drawing of the lateral surface of the cat's brain showing the location of EVA, the ectosylvian visual area (black area), on the ventral lip of the retracted anterior ectosylvian sulcus (ecsa). Numbers represent Horsley-Clarke planes. B: Coronal section cut at HC + 13 (see A) shows the location of EVA relative to a number of cortical areas. SVA: Splenial visual Area; 17, 18, 19: Areas 17, 18 and 19; AMLS (ALLS): Antero-medial (lateral) lateral supra-sylvian sulcus visual area; SF: suprasylvian fringe auditory area; Ep: posterior ectosylvian division of auditory cortex; AI and AII: Primary and secondary auditory cortex; SII: Secondary somato-sensory cortex; INS: Insular cortex; CL: claustrum. Sulci represented are: spl: splenial; lat: lateral; sups: suprasylvian; ecsa: anterior ectosylvian sulcus; sil: psudo-sylvian; rps: posterior rhinal.

A.


−10
0
+10
+20
+30
SF
AI
Ep
EVA
AII
INS

B.


+13
18
lat
17
19
spl
SVA
sups
AMLS
SF
AI
ALLS
AII
SII
ecsa
EVA
CL
INS
sil
rps

sectioning at 60µm on a freezing microtome. Sections were then reacted with tetra methylbenzidine (TMB) as the chromogen following the procedure of Mesulam (1978) (see Chapter 1). Sections were then mounted and coverslipped before being examined microscopically in light and dark fields. Reacted sections were photographed or drawn with the aid of a drawing tube before representative sections were stained with neutral red to distinguish laminae and cytoarchitectonic boundaries.

RESULTS

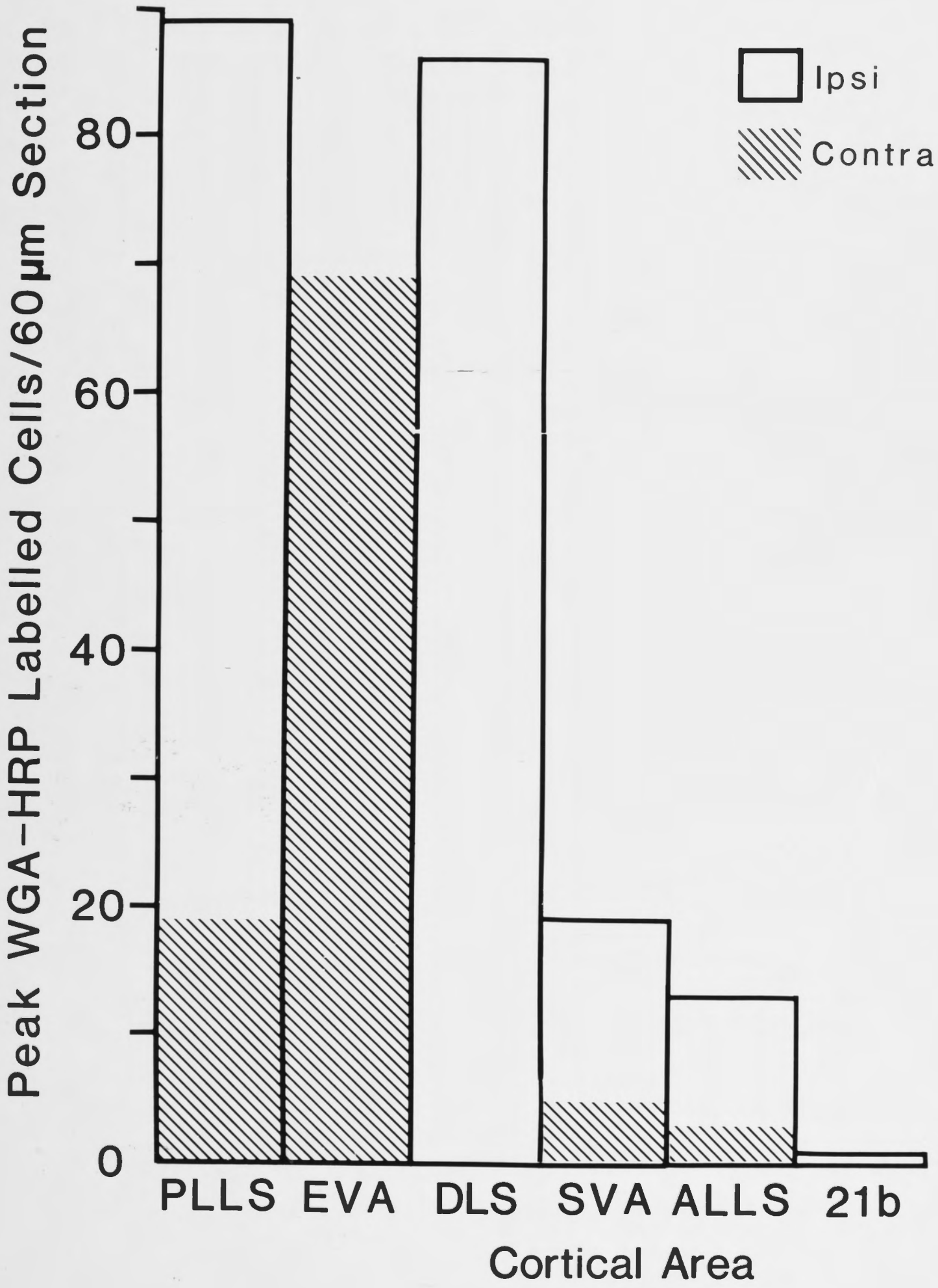
Following an injection of WGA-HRP into EVA it was possible, when viewing sections (60um thick) in the absence of counterstain, to observe labelled axons coursing through the white matter and also labelled cellls and terminals in those neural centres linked with EVA. In the case of retrograde labelling, the strength of the link from a given area was estimated from the incidence of labelled cells averaged over a selected number of sections which produced the highest count. The strength of the link demonstrated by terminal filling was not assessed beyond a heavy/light evaluation and it was not possible to separate the filled terminals of afferents from those of collaterals of retrogradely filled axons.

Visual cortical areas associated with EVA

In the homolateral hemisphere, four regions of visual cortex were found to contain appreciable numbers of labelled cells following the injection of WGA-HRP into EVA. In most of these locations, anterograde labelling of axon terminals also was apparent around the labelled cells. The terminal filling was usually coextensive with the spread of labelled cells and, in general, the higher the incidence of labelled cells, the greater the density of filled terminals.

The bar histograms in Fig. 2, drawn from a representative experiment, show the relative incidence of labelled cells in different regions of visual cortex after

Fig. 2. Relative numbers of labelled cells following injection of WGA-HRP into EVA. Counts averaged from the most densely labelled histological sections (60µm thick). PLLS (ALLS): Posterior (anterior)-lateral lateral suprasylvian sulcus visual area; DLS: Dorso-lateral sulcus visual area; SVA: Splenial visual area; 21b: Area 21b.

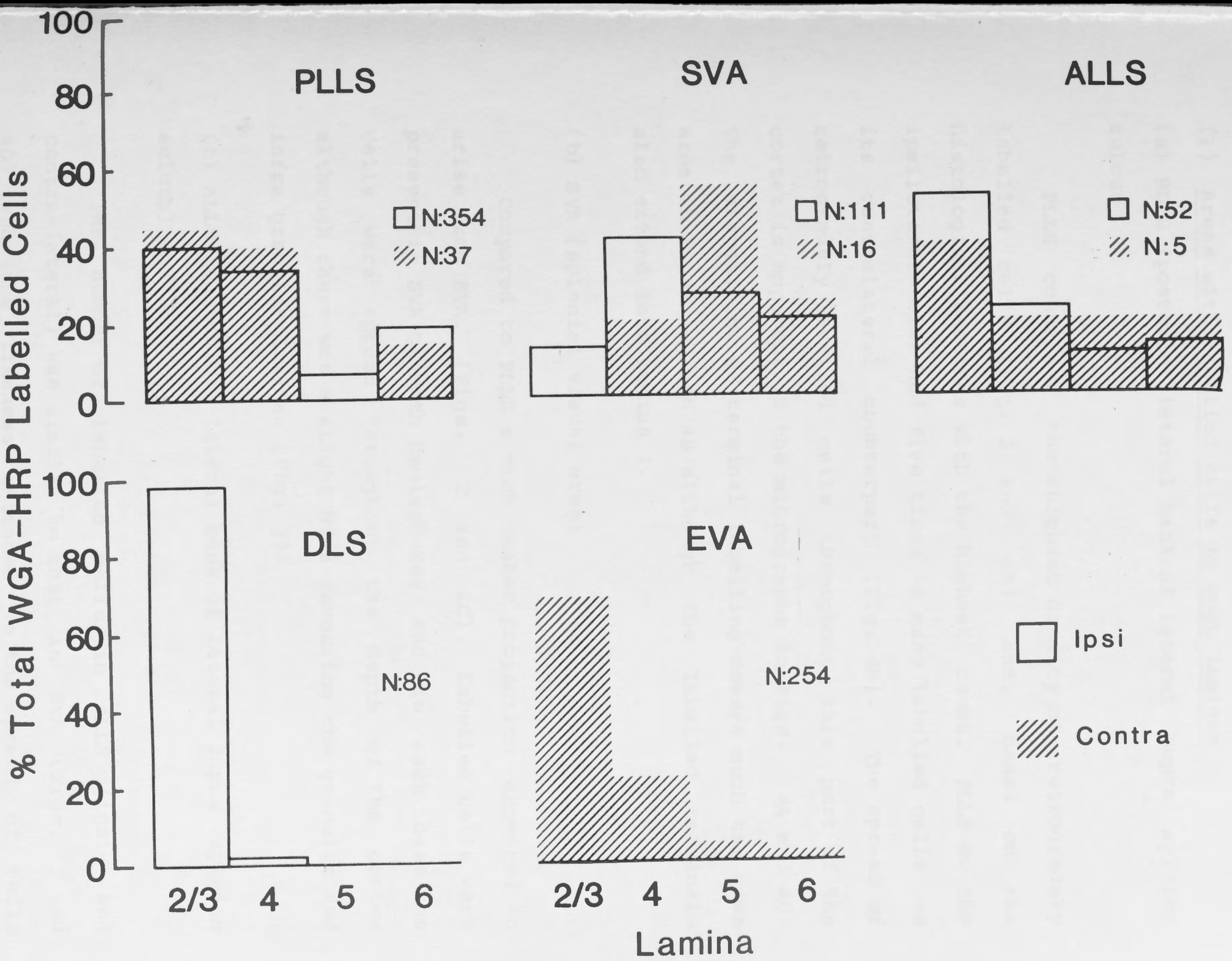

Peak WGA-HRP Labelled Cells/60µm Section
80
60
40
20
0
PLLS
EVA
DLS
SVA
ALLS
21b
Cortical Area
Ipsi
Contra

the injection of WGA-HRP into EVA. Regions in both hemispheres are represented in Fig. 2 (open bars: ipsilateral; hatched bars: contralateral) and the height of each bar registers the counts of labelled cells averaged over the most densely-packed sections (2 to 1Ø) from the region. The interpretation of the results in Fig. 2 is aided if it is appreciated that, with the exception of the splenial visual area (SVA), all of the visual regions with labelled cells lie lateral the lateral suprasylvian sulcus.

In a more detailed analysis in Fig. 3 laminar distribution histograms were prepared from counts of retrogradely labelled cells for the five areas of visual cortex. In Fig. 3 the open bars represent the proportion of labelled cells in each lamina of the designated area. Two broad patterns appear to be present in the distributions in Fig. 3. In the first (the top three histograms for PLLS, SVA and ALLS), labelled cells were found in almost equivalent proportions in most laminae. In the second type of distribution (the lower two histograms for DLS and contralateral EVA), few or no labelled cells were found in the infragranular layers and the greater part of the population was found in the supragranular laminae.

The following description of the results in individual cortical regions adopts the grouping in Fig. 3, so that regions in the first group had labelled cells scattered throughout the cortex while, in the second group, the cells were principally confined to the supragranular laminae.

Fig. 3: Laminar distributions of retrogradely labelled cells, following injection of WGA-HRP into EVA, expressed as percentage of labelled cells in given area. Abbreviations the same as in Fig. 2.


% Total WGA-HRP Labelled Cells
100
80
60
40
20
0
PLLS
N:354
N:37
SVA
N:111
N:16
ALLS
N:52
N:5
DLS
N:86
EVA
N:254
Ipsi
Contra
2/3
4
5
6
Lamina

(1) Areas with labelled cells in most laminae

(a) PLLS (posterior lateral bank of lateral supra sylvian sulcus

PLLS contained the highest density of retrogradely labelled cells (Fig. 2 and 4A) and, based on the histological sections with the highest count, PLLS on the ipsilateral side had five times as many labelled cells as its contralateral counterpart (Fig. 4B). The spread of retrogradely labelled cells throughout this part of the cortex is apparent in the micrographs in Figs. 4A and 4B. The disposition of terminal labelling covers much the same area in Figs. 4A and 4B although the labelled terminals also extend into lamina 1.

(b) SVA (splenial visual area)

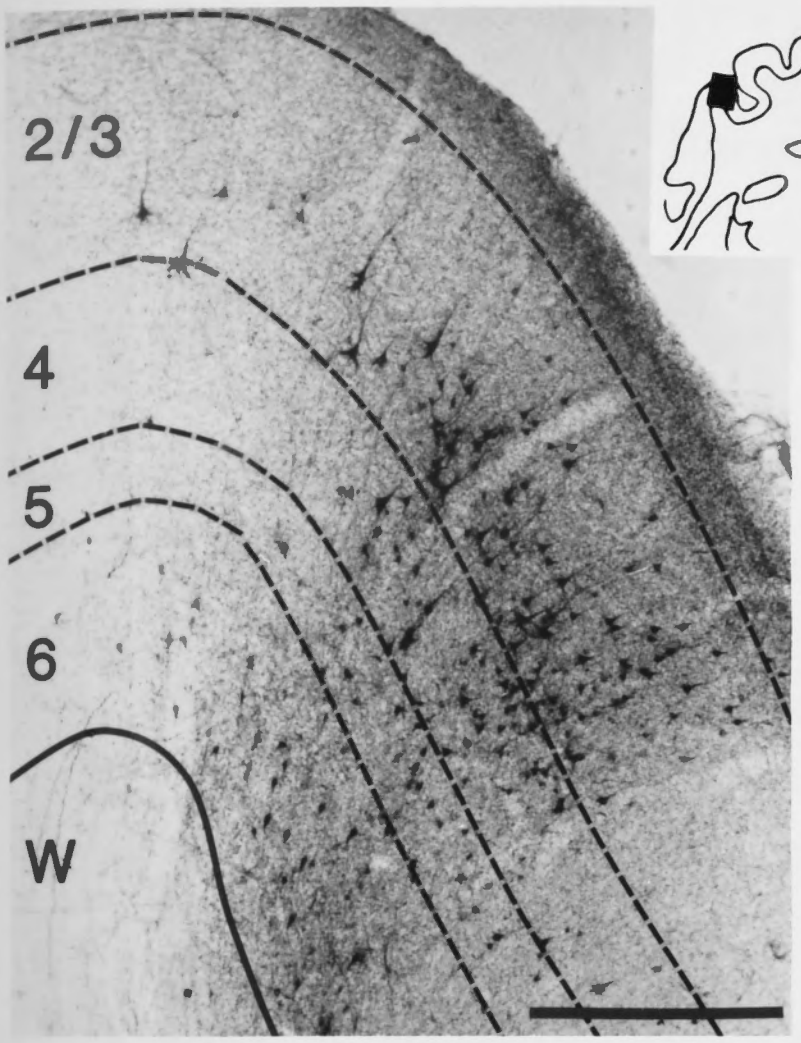
Compared to PLLS a much weaker projection appeared to arise from SVA. (Figs. 2 and 4C). Labelled cells were present in SVA of both hemispheres and in each case the cells were spread throughout the depth of the cortex although there was a slight bias favouring the granular and infra granular laminae (Fig. 3).

(c) ALLS (anterior lateral bank of lateral supra sylvian sulcus)

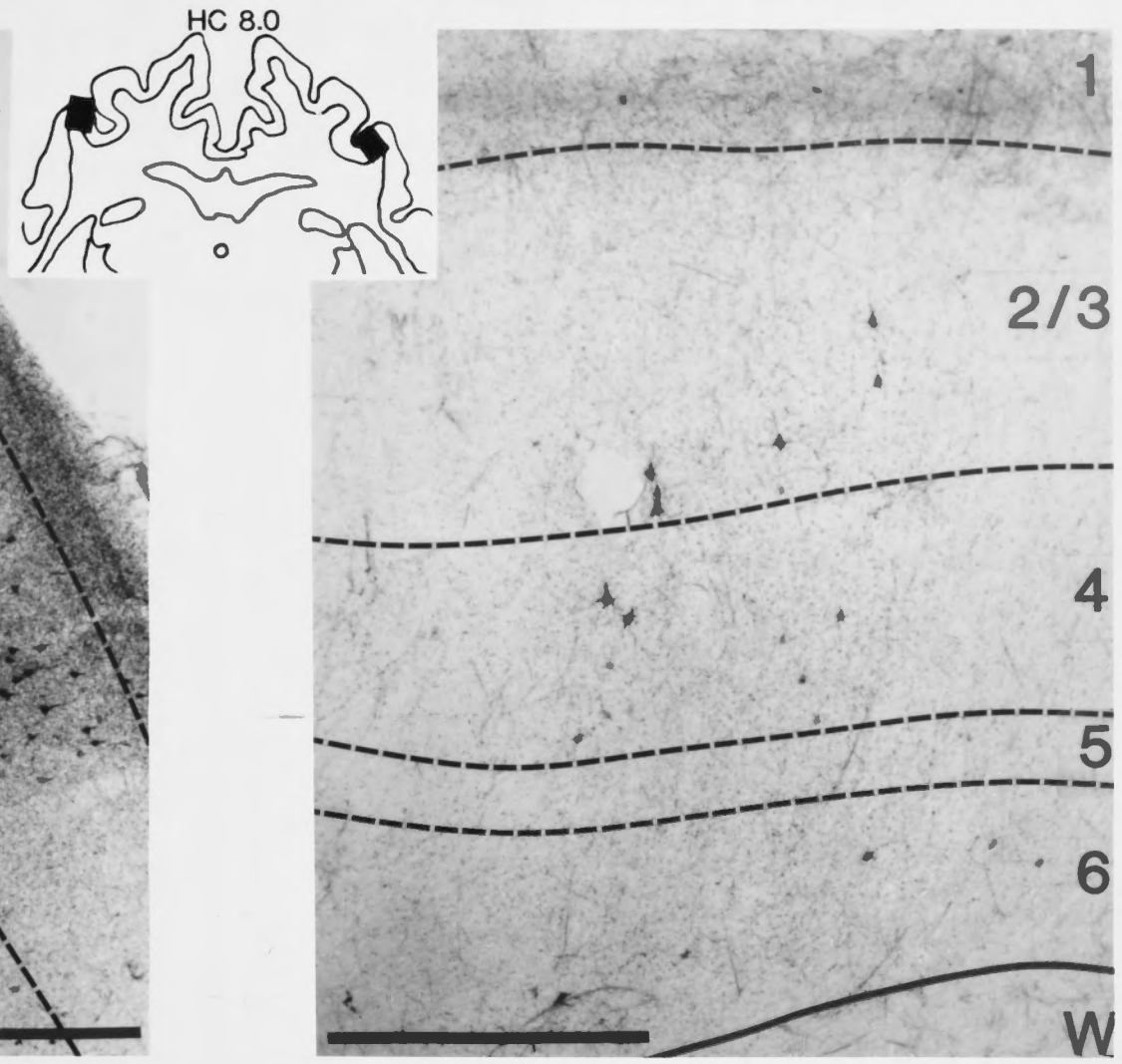
The number of labelled cells in ALLS (ipsi- and contra-lateral) was similar to that in SVA (Figs. 2 and 4D) and the likeness extended to the spread of cells throughout the cortex. A slight bias in ALLS, however, favoured the supra- rather than infra- granular laminae

Fig. 4: Representative bright field, micrographs of visual areas labelled cells projecting to EVA in areas where there was a relatively uniform spread across the cortex. Insets show location of each micrograph. Scale bars: 500 µm.

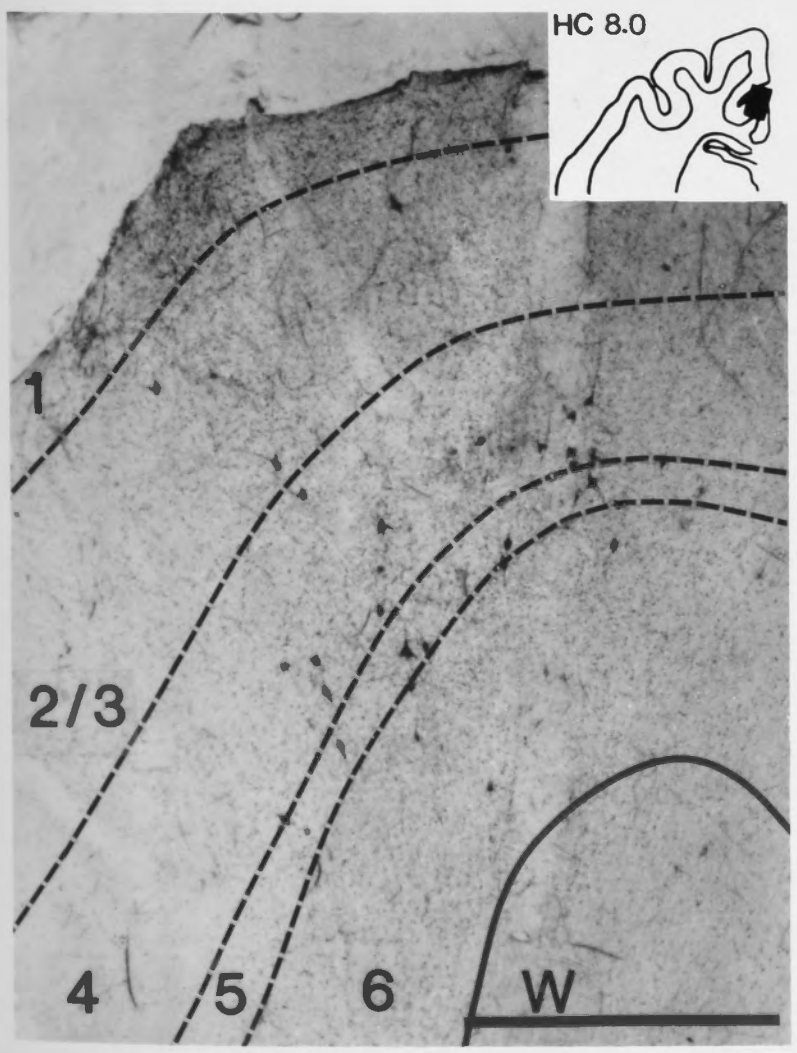
A. Area PLLS



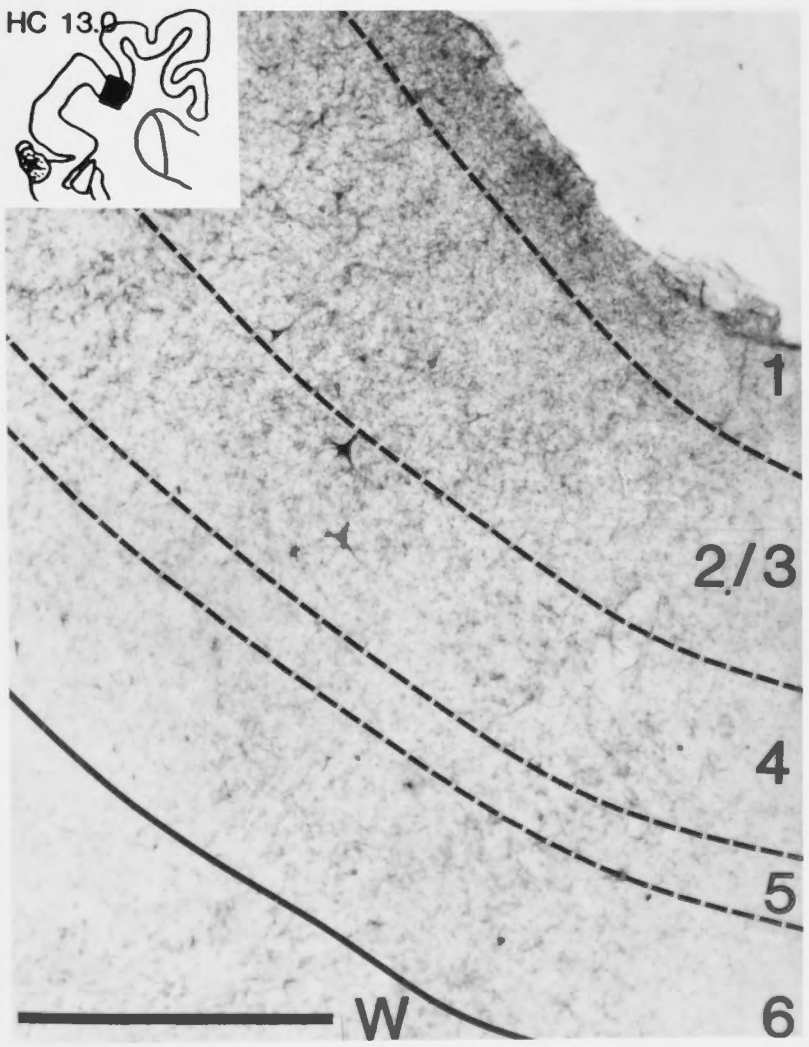
B. Area PLLS (contra)



C. Area SVA



D. Area ALLS



(Fig. 3).

(2) Areas with labelled cells in supra granular laminae

(a) DLS (Dorsal lateral supra sylvian sulcus)

Ipsilateral DLS had a high peak density of retrogradely labelled cells which, although similar in number to those in PLLS (Fig. 2), differed in that they were restricted to the supra-granular laminae (Figs. 3 and 5A). The labelling of DLS showed little sign of terminal labelling and there were no labelled cells in contralateral DLS.

(b) Contralateral EVA

The contralateral region with the highest count of retrogradely labelled cells was EVA (Figs. 2 and 5C) and here the count came close to that found in the regions of highest incidence on the ipsilateral side (viz. PLLS and DLS). Most of the labelled cells were in the upper cortex and only 5% were found in the infra-granular laminae. labelled terminals were also apparent in the upper laminae of contralateral EVA (Fig. 5c).

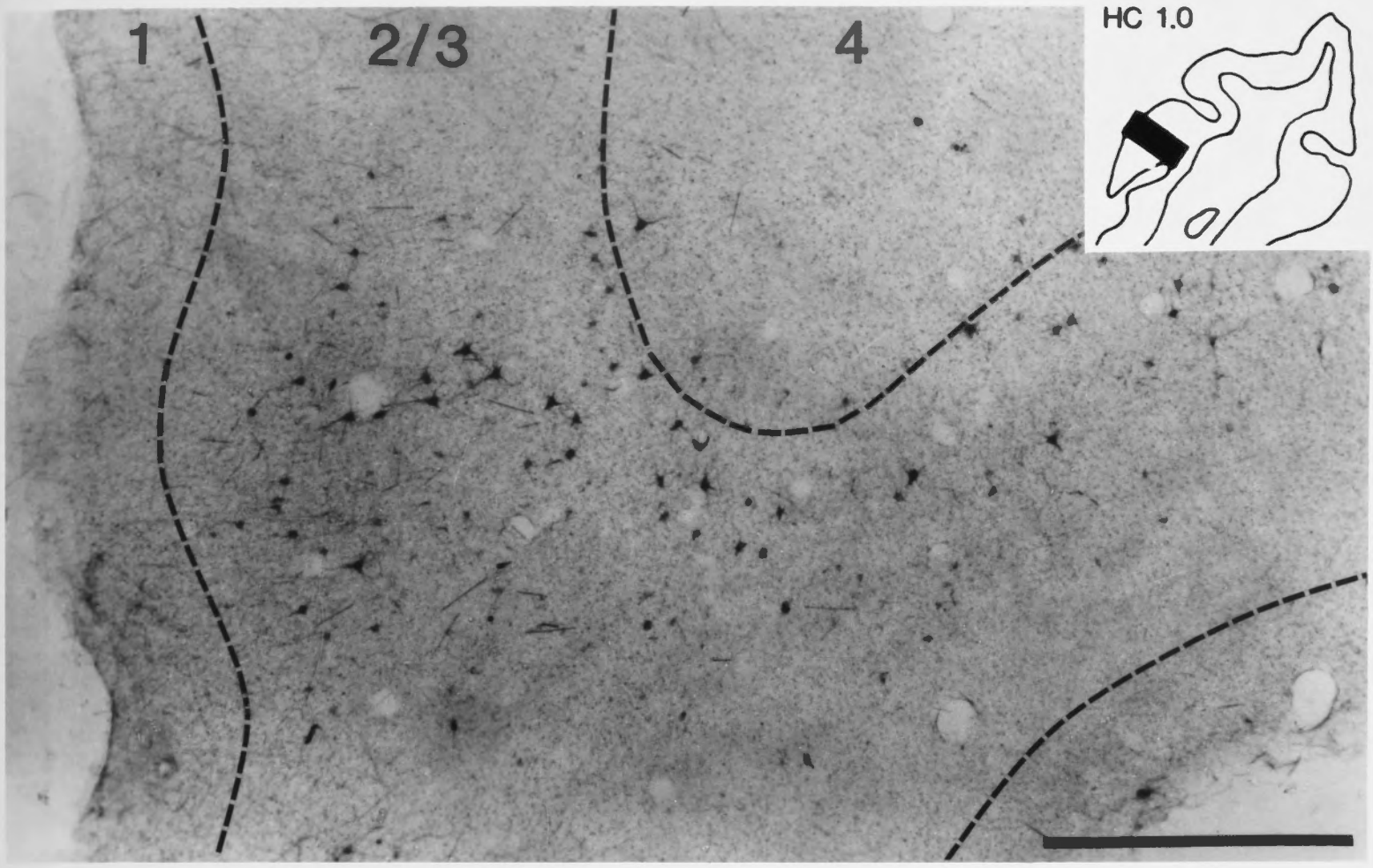
In addition to the five visual regions listed above, occasional labelled cells were also observed in Area 21b.

Axonal Pathways Associated with EVA

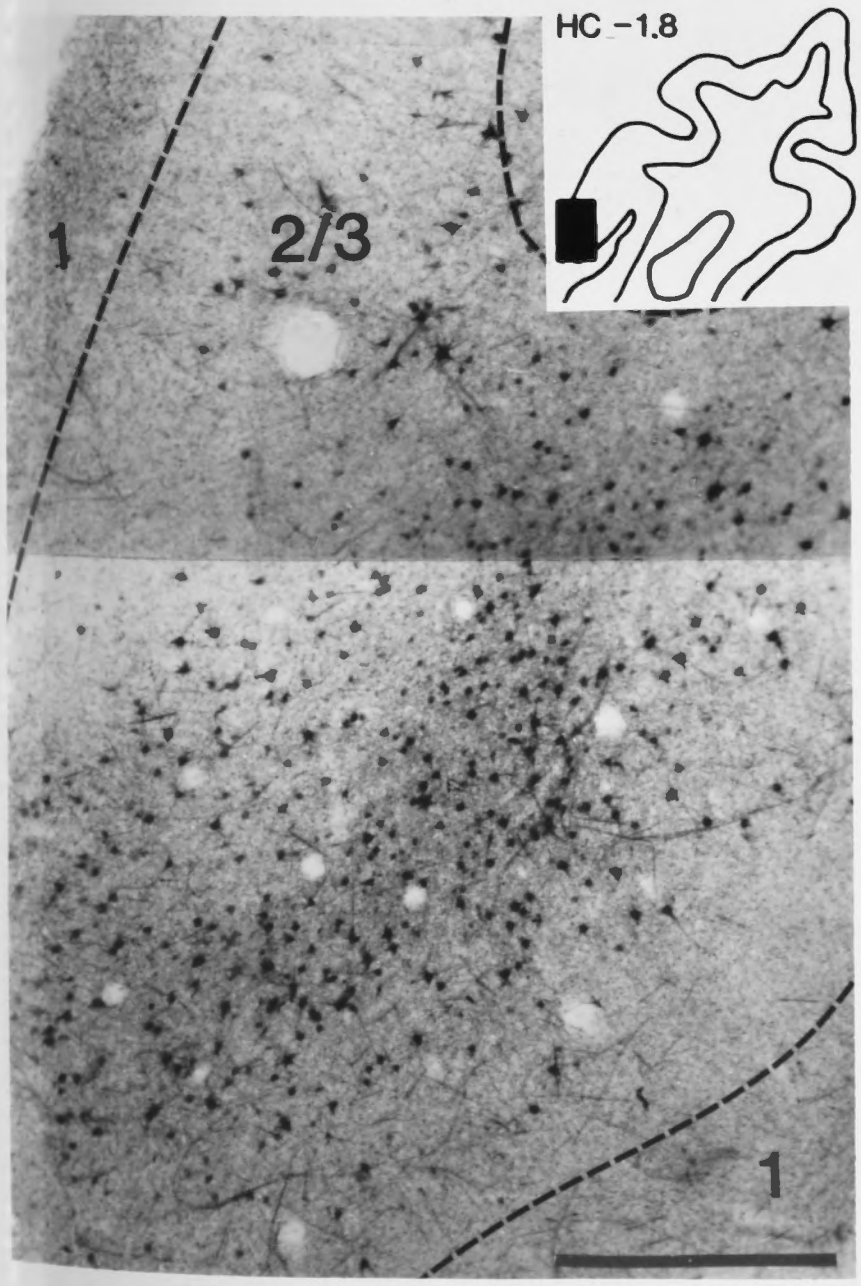
It had been noted in some earlier experiments (Boyapati et al., 1985), after the injection of WGA-HRP, that reaction with TMB revealed the intermediate course of axonal pathways. One could not be sure if this labelling

Fig. 5: Representative bright field, micrographs of visual areas with retrogradely labelled cells, projecting to EVA, where most cells were in supragranular laminae. Also included is a micrograph from the posterior ectosylvian auditory cortex (AUD) where labelled cells had a similar distribution. Inserts show location of each micrograph. Scale bars: 500 µm.

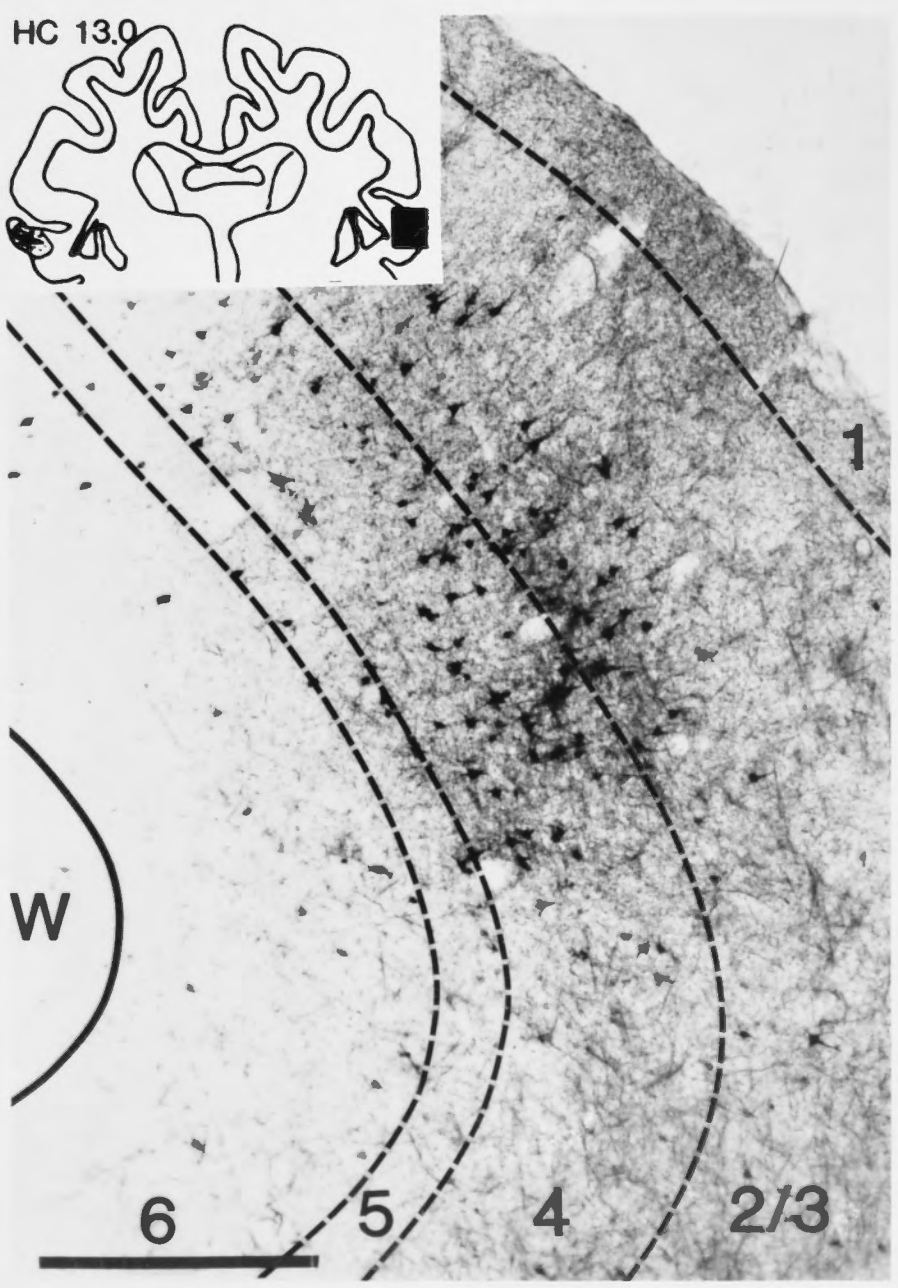
A. Area DLS



B. Area AUD



C. Area EVA (contra)



resulted from retrograde or antergrade transport or from both but the tracer tended to become indistinct towards the termination of the pathway and so favoured, without proving, the idea that the flow had been in an anterograde direction. If it is assumed that the labelled pathway eminated from EVA, then this projection broke up into three distinct sub divisions - one taking an ascending course and two following descending lines. Near the injection site in EVA, the pathway was dense and tightly packed and it retained this form as it passed in the extreme capsule over the dorsal tip of the claustrum. At this point, it became diffuse as it passed forward and upward, above the LGN. It reformed into a coherent band (Ø.25 x 3.Ø mm) at HC AP +8. In this form, the pathway extended upwards until it approached the fundus of the lateral supra-sylvian sulcus where fibres were seen entering the lateral bank of the sulcus.

At the lateral level of the lateral supra sylvian sulcus the pathway turned in the direction of the corpus callosum. In contra distinction to the claustral-cortical link (cf. Chapter 4 and Boyapati et al., 1985) no branches were observed entering the corona radiata at this point and there appeared to be a lack of direct association with the primary visual complex (Areas 17, 18 and 19). The pathway through the corpus callosum entered the contralateral hemisphere where its course was symmetrical to that taken on the ipsilateral side. The label finally became too weak to distinguish in the external capsule.

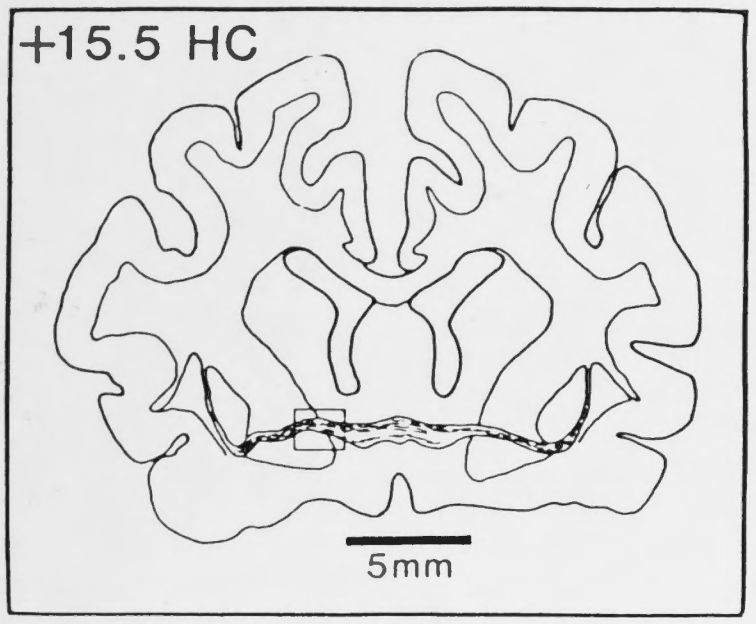
The first of the two descending branches departed from the ascending pathway as it passed across the dorsal surface of the claustrum. At this point, a group of labelled fibres could be seen descending into the external capsule where it passed between the claustrum and the adjacent putamen. The bundle passed anteriorly along this course until it emerged, at approximately AP+15.5, at the initial segment of the anterior commissure. Labelled fibres crossed the commissure (see Fig. 6) and developed a bilateral symmetry in the way in which they entered contralateral external capsule. The maintenance of this symmetry would mean that the heavy labelling in contralateral EVA could have been transported across this commissural pathway.

The second descending branch of labelled axons left the main stream of ascending fibres as they passed above the claustrum. The destinations of this group were difficult to follow, since the path quickly became diffuse as it descended forward towards the midbrain. Heavy anterograde and retrograde labelling in the thalamic nuclei (the medial segment of lateral posterior complex and the suprageniculate nucleus) (cf. Olson and Graybiel, 1983) suggested that these regions were the targets of this projection.

Summary of visual cortical links with EVA

Figure 7 adopts the diagrammatic representation, introduced by Symonds and Rosenquist (1984a), to indicate the connectivity of EVA with regions in the ipsi- and

Fig. 6: Dark field micrograph of interhemispheric pathway (white lines) through the anterior commissure revealed after the injection of tracer into EVA. Inset shows location of micrograph.



Anterior

commissure

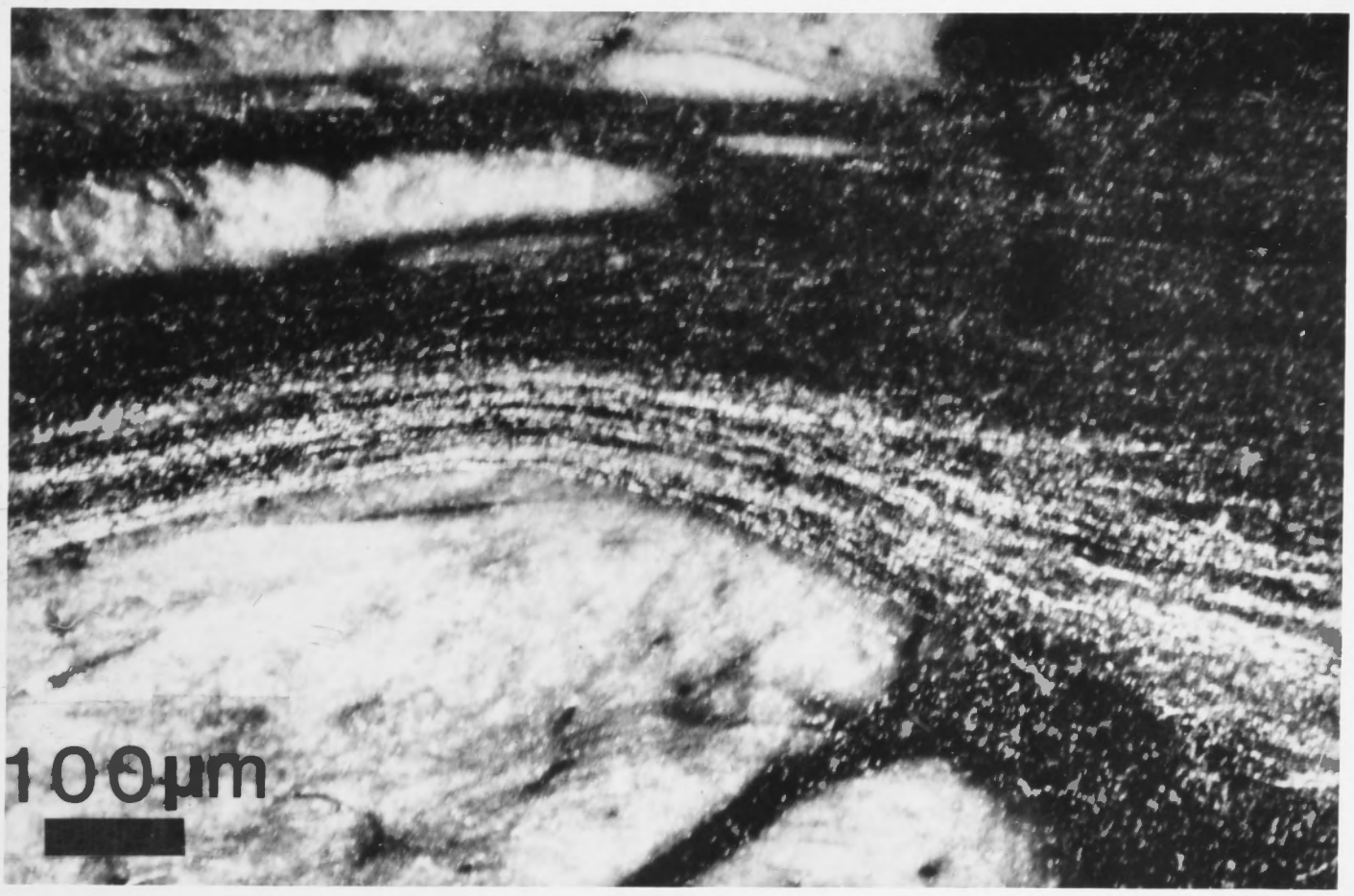
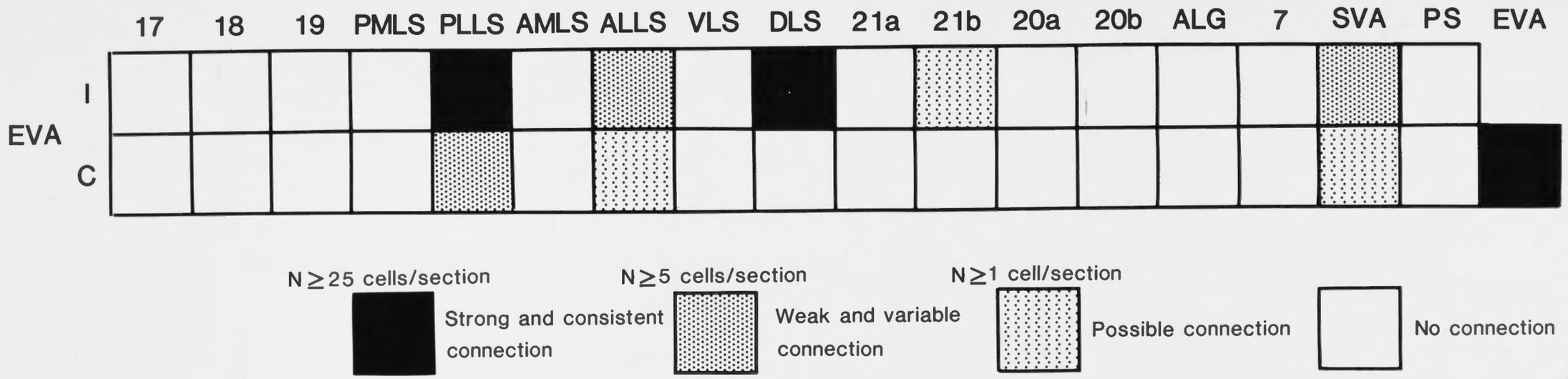


Fig. 7: Summary diagram showing the bi-lateral connectivity of EVA. Criteria for strength of link taken from Symonds and Rosenquist (1984a).


17
18
19
PMLS
PLLS
AMLS
ALLS
VLS
DLS
21a
21b
20a
20b
ALG
7
SVA
PS
EVA
I
EVA
C
N ≥ 25 cells/section
Strong and consistent connection
N ≥ 5 cells/section
Weak and variable connection
N ≥ 1 cell/section
Possible connection
No connection

contra-lateral hemispheres (I and C). Linkage with EVA has been divided into strong (more than 25 labelled cells/section) weak (5 to 25 labelled cells/section) and possible (1 to 5 cells/section) categories. This representation shows that the major ipsilateral visual links with EVA were with PLLS and its neighbour DLS, while the principal contralateral association of EVA was with its namesake on the opposite side. The auditory area with the strongest link was the Ep region which, in turn, was not far removed in strength to that from PLLS and DLS.

Non-visual cortical areas associated with EVA

All regions in the auditory cortex (AI, AII, SF and Ep) had labelled cells indicating the presence of projections to EVA. The highest incidence of labelled cells was found in the ectosylvian division, Ep, where the counts were similar to those areas of densest labelling in the visual cortex (ie PLLS and DLS). Similar numbers of labelled cells were also found in the somato-sensory cortex, SII, and in the insular cortex. The laminar distributions of relay cells in these non-visual areas varied from being confined largely to supra-granular laminae in Ep and to being in the infra-granular laminae in the insular cortex.

DISCUSSION

Ipsilateral Cortical Links With EVA

The findings of the present study largely confirm the neural connectivity of EVA described in earlier studies (Olson and Graybiel, 1981; Mucke et al., 1982; Symonds and Rosenquist, 1982a), although there are some noteworthy differences in detail. All previous reports described a strong reciprocal link between EVA and the lateral division of the Clare-Bishop complex. This link becomes stronger posteriorly along the lateral lip of the lateral suprasylvian sulcus (from ALLS to PLLS), but it is its extension beyond PLLS that provides the greatest point of divergence in the various accounts. Olson and Graybiel (1981) described linkages extending into Area 2Ø while in the present study, it was observed the extension rather into DLS (also observed by Mucke et al., 1982) and also into Ep of the auditory complex: two regions that are adjacent to the strongly linked PLLS. With one exceptional link (that to SVA), the results confirmed the previous findings that EVA had no connection with visual regions lying medial to the lateral suprasylvian sulcus (cf. Mucke et al., 1982, who reported a link between EVA and PMLS). Earlier reports have not described the connection with SVA. If attention is confined to the strongest linkages, however, there is general agreement that EVA makes its principal tie with the posterior section of the lateral suprasylvian sulcus. The reasons for differences in the description of secondary regions linking with EVA are not obvious, but they may result from small variations in the

placement of the tracer and consequent differences in involvement of regions adjacent to EVA.

Contralateral Cortical Links with EVA

The present study provides new material on the links that EVA makes with the contralateral hemisphere. All cortical regions with an ipsilateral link, with the exception of DLS, had corresponding contralateral regions that connected with EVA. The strength of the contralateral link was much weaker than its ipsilateral counterpart (on average only a quarter of the number of labelled cells). By far the strongest contralateral connection (three times greater than the next most heavily labelled) passed between EVA and its homologue on the opposite side. This linkage appears to supply the morphological substrate for the visual response properties ascribed for single cells in EVA (Olson and Graybiel, 1981; Mucke et al., 1982). That is, the high incidence of binocularly driven cells and receptive fields that extended over the vertical midline into the ipsilateral hemifield may be achieved through signals passing from one EVA to the other. The path for this interchange may travel either through the corpus callosum or via the anterior commissure (c f. Micele et al., 1984) both of which appeared to be passing towards the contralateral EVA when lost in the underlying white matter.

A binocular exchange at this point, however, appears to occur at a very late stage in the visual processing sequence since EVA is only linked indirectly, through PLLS, to areas of primary reception in the visual cortex. It

would be surprising in this line, therefore, given the high incidence of binocular units in Areas 17, 18 and 19, if binocularity were not already established before the signal arrived at EVA. Similarly, a second afferent pathway coming to EVA from the tecto-receiving zone in the medial segment of the lateral posterior nucleus of the thalamus (Berson and Graybiel, 1983) is likely to carry binocular information since a high proportion of cells in the superior colliculus can be driven from either eye (Sterling and Wickelgren, 1969). There is one feature, however, that may be exclusive to the binocularity expressed in the responses of EVA cells. This is the extension of many receptive fields 10-20° into the ipsilateral hemifield, which seldom occurs in binocular units in other regions and could be related to the contribution that comes with the interhemispheric link at EVA.

Laminar Distribution of Cortical Cells Projecting to EVA

From the initial work of Gilbert and Kelly (1975) it has been concluded that cells, contributing to the forward progression from one visual cortical region to the next, reside in the supragranular laminae, whereas cells subserving a feed-back modulating role reside in infra granular laminae. Bullier et al. (1984) have sought to modify this proposal by suggesting that infra- and supra-granular cells may both contribute to cortico-cortical projections (see also Symonds and Rosenquist, 1984b), and that the proportion of each depends on the position occupied by the area in the cortical

on the position occupied by the area in the cortical sequence. Thus, in Areas 17 and 18 most of the cells initiating cortico-cortical projections reside in the supragranular laminae while in Area 2Ø, which lies presumably towards the end of the sequence, the cortico-cortical projections arise from infragranular laminae. At areas located between the two extremes, say area 19 or PMLS, cells contributing to cortico- cortical pathways occur in equivalent numbers throughout infra- and supra-granular laminae.

With the scheme outlined above, the laminar distributions observed in ALLS, PLLS and SVA, with equivalent infra- and supra-granular involvement, (See Fig, 3) would lie mid-way along a given processing sequence while DLS with a predominance of supragranular projection, would occur much earlier in, what is presumably, a different processing sequence. The accuracy of this interpretation clearly depends on the extent to which cells of origin in intercortical pathways are removed to the infragranular laminae with progression along the processing sequence. In a complex system where there may be criss-crossing pathways, it is difficult to identify the sequential location of an area and equally difficult to test the validity of clues about the sequence that may come from differences in the laminar pattern. The present results do little to resolve the question of EVA's position in the processing of visual information and this will continue to be the case until more is known of the functional changes that accompany the step from each relay

Possible Polysensory Role of EVA

The strong projection from the auditory cortex (AI, AII, SF and Ep) and from the somato-sensory cortex (S11) and the insular cortex suggests that EVA may subserve a synesthetic role. Squeezed between insular cortex on the dorsal bank of the lateral sulcus on the one hand, and primary (AI) or secondary auditory cortex (AII) on the dorsal bank of the ectosylvian sulcus, on the other (see Fig. 1), EVA appears particularly well situated to participate in the integration of auditory and visual information.

The insular cortex itself receives input from a wide variety of cortical regions, including visual areas which can be identified with 19, PMLS, PLLS, ALLS, VLS, SVA, PS and 21b, as well as auditory areas such as the suprasylvian fringe area (SF), area Ep, and both AI and AII (Guldin and Markowitsch, 1984). In addition to projecting to EVA, the insular cortex sends axons to ALLS and to the auditory areas, AI, AII, Ep and SF (Cranford et al., 1976). The relationship that EVA has with the auditory and insular cortex, suggest that EVA may have a role in mediating polysensory phenomena such as "visual capture", despite the negative result of Mucke et al. (1982), who were unable to evoke gross auditory or somasthetic responses from neurons in EVA. In attaining the visual localization required for visual capture, the cells of EVA would benifit from their own binocularity and their close link with the lateral

division of the visual cortex. This area, like EVA, is subserved by the tecto-receiving zone of the thalamus and this association open the way to the mid-brain centres responsible for eye movements. With its mid brain associations, its dense interhemispheric and auditory linkages, EVA could have a role in the merging of binaural and binocular localization.

In addition to raising interesting points on the functional role of EVA the present study also fulfilled its original aim of establishing the signs to indicate contamination of EVA when label was being injected into the claustrum. In particular, the presence of labelled cells in DLS was adopted as a danger signal although DLS labelling was not observed in any of the four experiments involving the injection of tracer into the claustrum.

CHAPTER 5

THE CHARACTER AND INFLUENCE OF THE CLAUSTRAL PATHWAY TO THE STRIATE CORTEX OF THE CAT

INTRODUCTION

The general description of the claustrum and its wide connectivity with other visual areas is presented in Chapter 4 and attention in this chapter will be confined to a consideration of the functional contribution that the claustrum makes to the striate cortex.

The response properties of the claustral neurons in the visual segment resemble those of cells in the visual cortex. There are distinctive features in the responses of claustral neurons, however, and LeVay and Sherk (1981a) found that most of them had a preference for comparatively long stimuli and, in general, lacked direction selectivity. Neither the velocity of stimulus movement nor the width of the bar was a critical feature. The majority of claustral cells were driven equally well from either eye (Creutzfeldt et al., 1980; Sherk and LeVay, 1981b).

In the past attempts to discover the functional role of the claustrum have often depend on the effects of selective destruction of the nucleus. Unfortunately, due to location of the claustrum, it is difficult to achieve its exclusive removal either in experimental conditions or as the aftermath of a pathological process. Without the results from selective ablation, the contribution of the claustrum has gone largely undefined.

Recently, however, Sherk and LeVay (1983) have made a more refined attempt to selectively remove the claustrum and evaluate its contribution. The logic of their

experiment was to sample the response properties of single striate neurons before and after the destruction of claustrum by injecting the neurotoxin, kainic acid. End-stopping or the striate cell's preference for fore-shortened stimuli, was measured qualitatively before and after the destruction. It was found that, there was a significant reduction in the proportion of cells with moderate end-stopping in their receptive fields. Similar observations were made in both simple and complex cells and Sherk and LeVay concluded that the claustrum contributed end-zone inhibition to bring the property of hypercomplexity to the responses of striate neurons. Since hypercomplexity has long been associated with form vision (Hubel and Wiesel, 1965), this appears to be the first indication that a nucleus, removed from the primary afferent pathway, might have a major influence of the pattern detecting system of the cat.

In the experiments involving the destruction of the claustrum, there was no evidence that the recorded cells in the striate cortex were linked with the claustrum. Anatomical experiments (autoradiographic studies) (LeVay and Sherk, 1981a) had shown that the major input to the striate cortex terminated in laminae 3 and 4 in regions representing the peripheral visual field (more than 5^{o} from the area centralis) and LeVay and Sherk directed their electrodes towards these regions. Whatever the strength of the input, however, a risk remained that the sampled cells would not be linked with the claustrum and that the recorded effect of the claustral influence would be diluted

accordingly. The present study overcomes this problem by confining the recorded sample to those striate neurons with a demonstrable drive originating from the claustrum.

The AIM of the present study

The existence and the nature of the claustral link with a recorded striate neuron can be established from the cell's response to electrical stimulation in the visual segment of the claustrum. Antidromic and orthodromic responses reveal if the striate neuron has outgoing or incoming link with the claustrum. In addition, the response latency to electrical stimulation gives an indication of the signal conduction times in these pathways and thus, the relative times of arrival of natural signals travelling to and from the claustrum.

The present study made a quantitative examination of the visual responses of striate neurons with a claustral link in search of specific features that may distinguish the functional role of these cells. At the same time, by observing the response to electrical stimulation, it was possible to characterize the axons contributing the claustro-striate pathway.

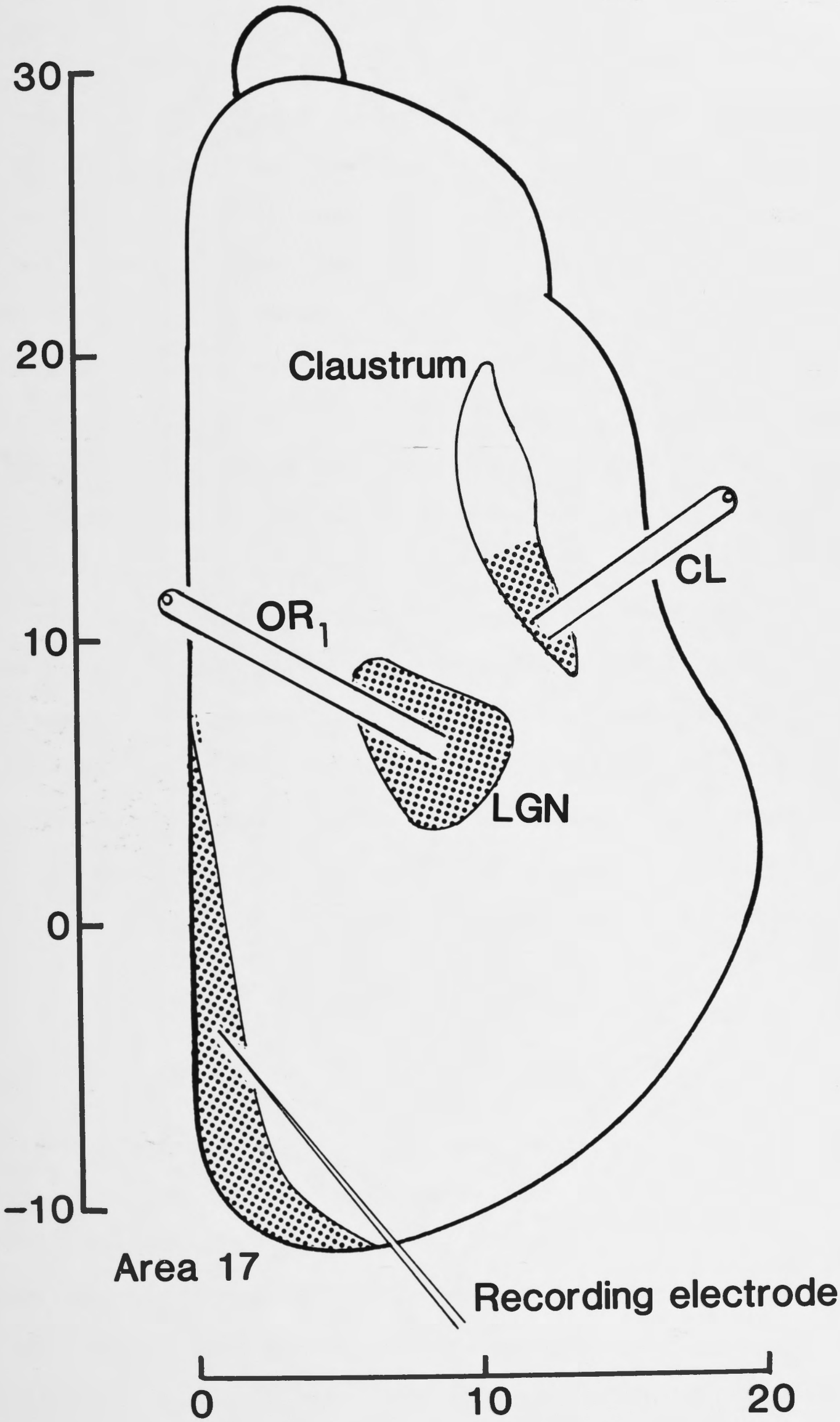
METHODS

The nine cats used in these experiments ranged in weight from 2.0-3.5kg. The general procedure was similar to that of used in Chapter 3. Single units were isolated with a Merrill-Ainsworth (Merrill and Ainsworth, 1972) tungsten-in-glass microelectrode (exposed tip around 10µm in length). Stimulating electrodes were placed in the visual segment of the claustrum (CL) close to HC AP 11.5; ML 12.0 at a depth of 12mm from the cortical surface and in the optic radiation (OR_1) close to HC AP 7; ML 9 at a depth of 10mm (Fig. 1). The precise position of the stimulating electrodes was determined by advancing a multi unit recording electrode (with a larger exposed tip) until units were encountered that were retinotopic correspondence (cf LeVay and Sherk, 1981b) with those recorded in the striate cortex. The stimulating electrodes were then directed as closely as possible to these locations.

Twenty five of the total sample of claustral driven striate neurons were subjected to a battery of tests to obtain a quantitative analysis of their visual response properties. For most of these cells the following measurements were made.

a) the receptive field dimensions
b) the velocity tuning curve
c) the orientation tuning curve
d) the ocular dominance value
e) the length response curve and
f) direction selectivity to moving bars.

Fig. 1: Schematic representation looking from above onto the right hemisphere showing relative positions of the stimulating electrode, OR_1 in the LGN, CL in the claustrum, and the recording electrode on the medial bank of Area 17. Scales show Horsley-Clarke co-ordinates. Stippling indicates relevant areas with visually responsive cells.


30
20
10
0
−10
Claustrum
CL
OR1
LGN
Area 17
Recording electrode
0
10
20

RESULTS

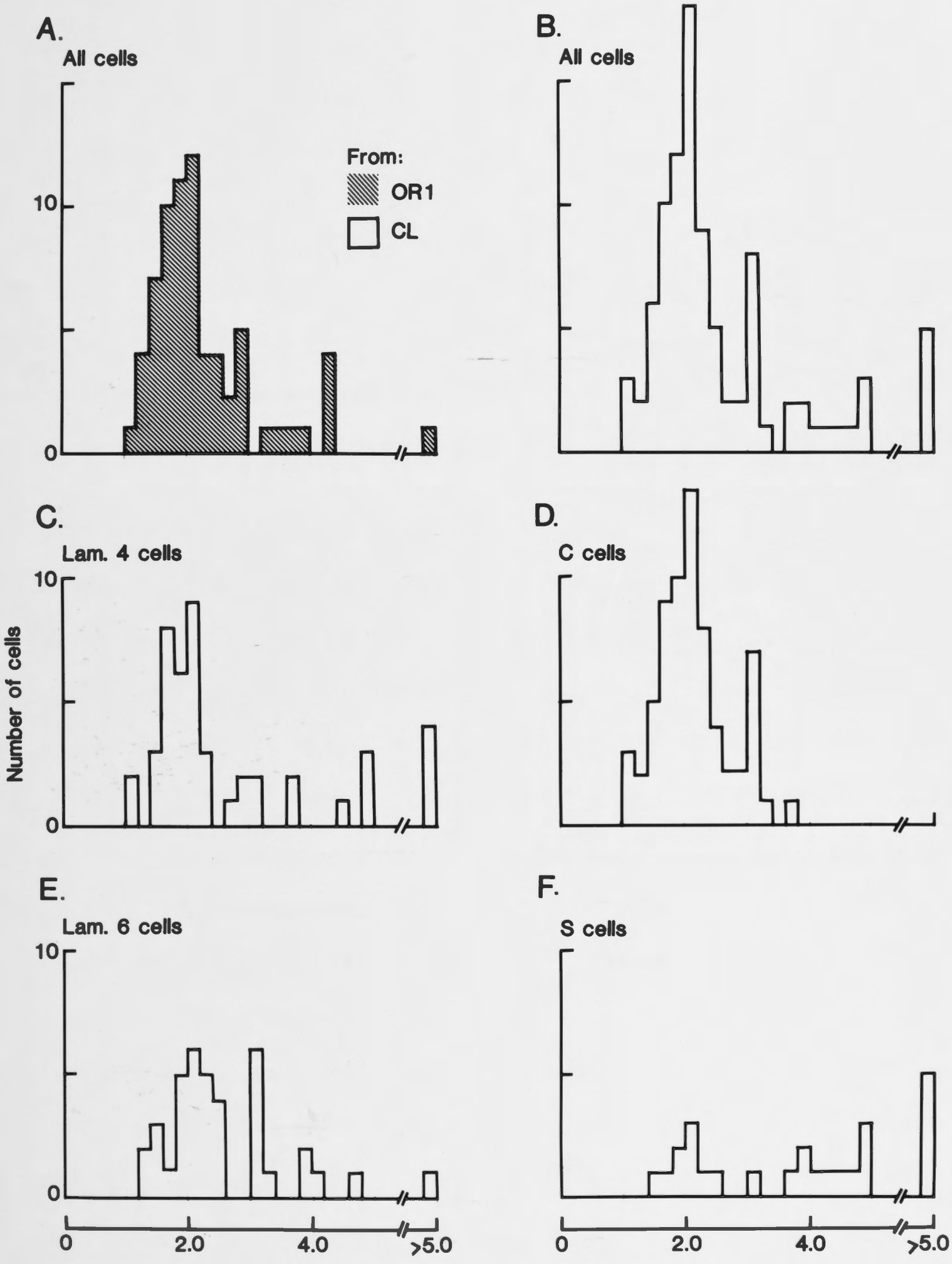
From tracer experiments in the cat, the outgoing pathway from the striate cortex to the claustrum appears to be smaller than its reciprocal. The striate neurons projecting to the claustrum make up only 3%-10% of the cells in lamina 6 (LeVay and Sherk, 1981a; McCourt et al., 1985). Consistent with this sparseness, only a single striate neuron was encountered that displayed an antidromic drive (OR_1 latency = 1.6ms; CL latency = 1.2ms) from the claustrum. This neuron had a non-oriented receptive field. In contrast, 11 penetrations produced 113 striate neurons that received an orthodromic or trans-synaptic drive from CL, the claustral stimulating electrode. It was these trans-synaptically driven cells that provided the data for analysis. The study was selective in that 96% of the classified cells had receptive fields within 5^{o} of the midpoint of the area centralis. For comparative purposes, more information is available in the general population for cells with central rather than peripheral receptive fields.

I. ELECTRICAL STIMULATION

Signal conduction in claustro-striate pathway

Looking for peaks that may indicate the ordinal position of claustral-driven cells (cf. Bullier & Henry, 1979a), Fig 2A was prepared to show the distribution of trans-synaptic response latencies for stimulation in the optic radiations at OR_1. Figure 2B then allows a comparison of this distribution with the spread of

Fig. 2: Distribution histograms of trans-synaptic response latencies in striate neurons following stimulation at OR_1 (A) and CL (B). The composition of B is shown in succeeding histograms which display the CL latencies for cells in lamina 4 (C); the cells in lamina 6 (E); for C cells (D) and for S cells (F).


A.
All cells
10
0
From:
OR1
CL
B.
All cells
C.
Lam. 4 cells
10
0
Number of cells
D.
C cells
E.
Lam. 6 cells
10
0
F.
S cells
0
2.0
4.0
>5.0
Trans synaptic latency (ms)

claustral or CL latencies for the same cells. For the sample of claustral-driven cells, the distribution of OR_1 latencies in Fig 2A was almost the same as the one presented previously for the general population of striate neurons (Bullier and Henry, 1979a) and, as Fig. 2B shows, there was a corresponding profile in the distribution of CL latencies.

In addition, Fig. 2 also shows the make up of the total population of CL latencies. Thus, Fig. 2C, shows the latency distribution for cells in lamina 4; 2E, for cells in lamina 6; 2D, for C cells and 2F, for S cells.

If the CL latency provides a guide to the ordinal position of claustral driven cells, then the large proportion of cells, in the first peak in Fig. 2B (with a CL latency of 2.5ms or less and a mean of 1.9ms) points to a high encounter rate of directly driven cells from the claustrum. With a more rigorous criterion of 2.0ms or less (cf. the criterion for OR_1 of 1.8ms or less used to indicate the existence of a monosynaptic input in the shorter geniculo-striate pathway by Bullier & Henry, 1979b) then 45% of the encountered CL driven cells still met the requirements for a direct CL drive. Of this group, as many as 80% also had the small latency variation (less than 0.5ms) to repeated stimulation, expected of directly driven cells (Bullier & Henry, 1979a).

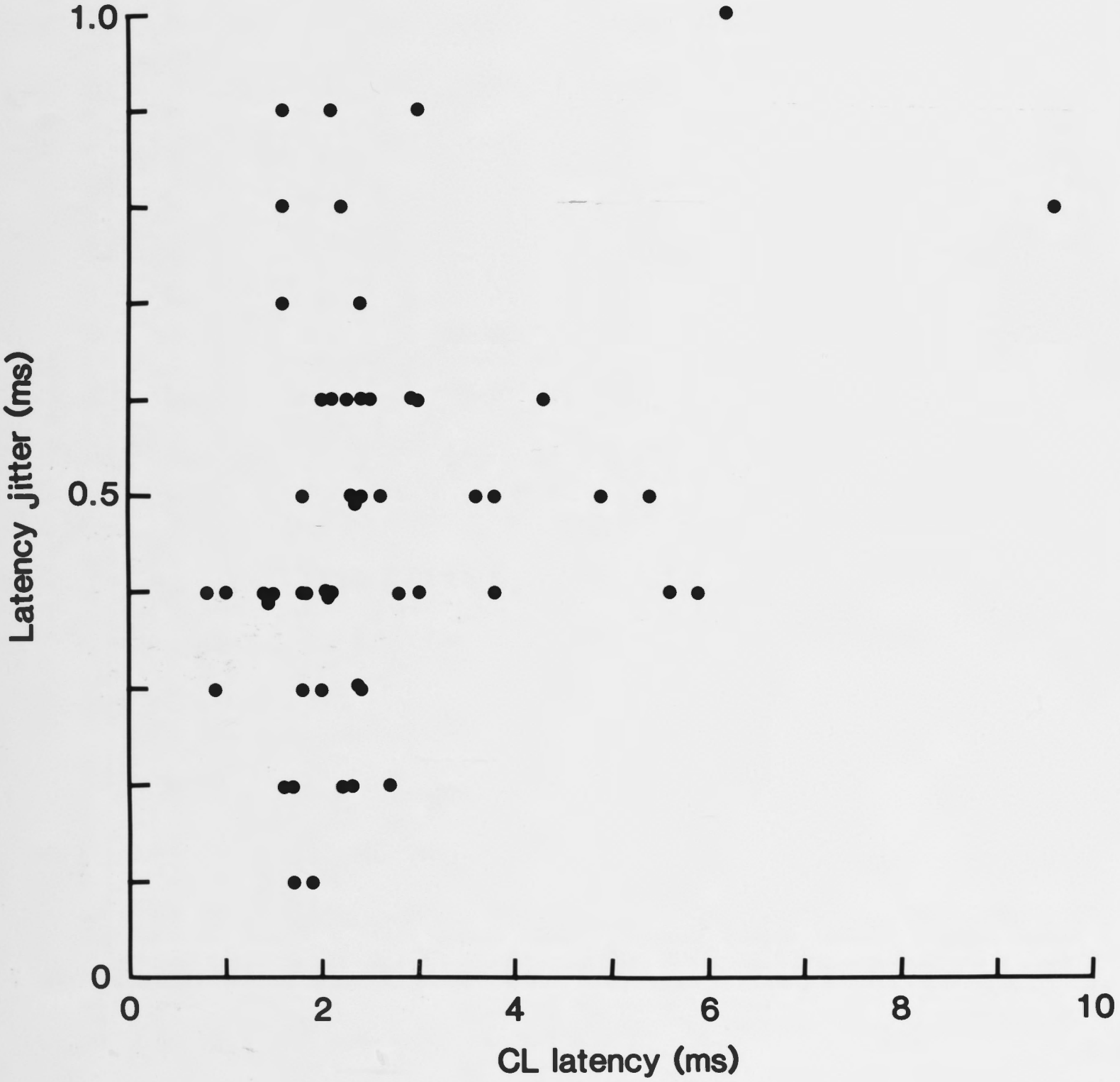
The comparison of CL latencies between cells in lamina 4 and 6 (Fig. 2; C&E) shows that the two groups had a similar distribution pattern. The possible distinction

that might come if the signal passed back along striate efferents and then via collaterals into lamina 6, but not into lamina 4 (see LeVay & Sherk 1981a; Katz et al., 1984), was not apparent as a difference in the two distribution curves.

When the cells were separated into their functional classes, it was found, as shown in Fig. 2 D&F, that there were 55 C cells (mean latency = 1.84ms) and 9 S cells (mean latency = 1.94ms) represented in the first peak. Fewer cells occurred outside the first peak and these appear to fall into two groups; the first, contained 13 C cells with CL latencies greater than 2.5ms (mean latency for the group was 3.0ms) and the second, 16 S cells with CL latencies of 3.0ms or more (mean latency = 5.4ms). With these longer latencies there was an uncertainty whether the signal delay was caused by slow axonal conduction or by the crossing of a number of intermediate synapses.

An alternative to the CL latency as a guide in assessing a cell's ordinal position was observed by noting how precisely repeated trans-synaptic responses follow the same time course. The level of latency variation or jitter appears to increase with each synaptic crossing and a low level of jitter (less than 0.5ms) may, therefore, be taken as the sign of a mono-synaptic drive (Bullier and Henry, 1979a; Fester and Lindstrom, 1983). The jitter associated with drive in the claustral pathway was plotted in Fig 3, against the shortest CL latency in the cluster of responses.

Fig.3: A scattergram showing the variability in the timing of the responses in individual striate neurons following repeated stimulation in the claustrum. The spread in these responses (or jitter) is plotted against the shortest latency in the cluster.


1.0
0.5
0
Latency jitter (ms)
0
2
4
6
8
10
CL latency (ms)

In a restricted sample, since precise jitter measurements were not made in some of the earlier experiments, most of the cells had a low response jitter. Fig 3 shows that responses in 36/53 cells driven from the claustrum the jitter was Ø.5ms or less. While it may be argued that the jitter duration is an inconclusive guide to ordinal position, the frequency with which a low level of jitter was observed in the response suggested that many cells received a direct input from the claustrum.

The intermediate course of the claustro-striate pathway

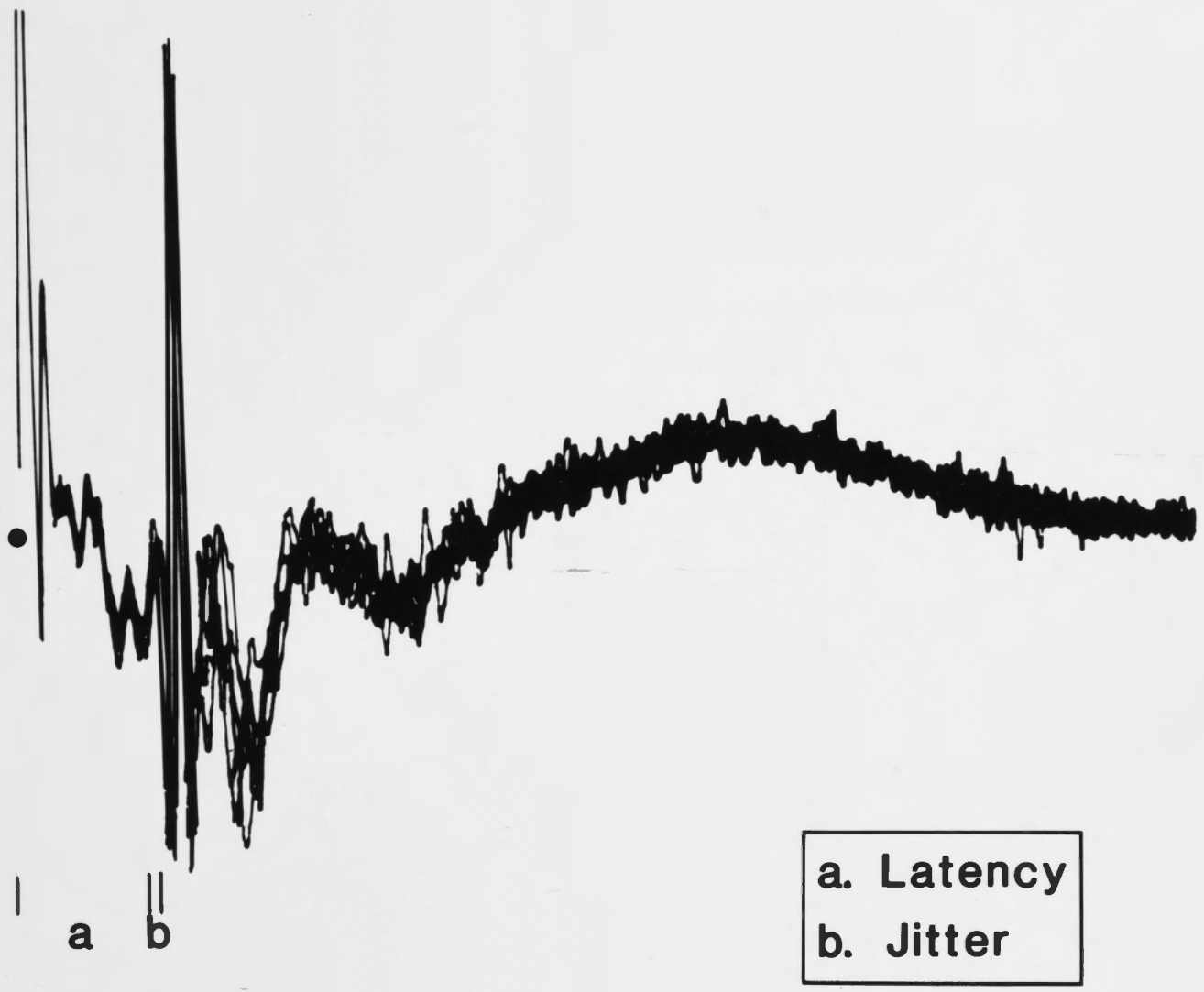
The intra-cranial course taken by the claustro-striate pathway has been described in Chapter 4. Although, the pathway only appears to come close to the geniculo-striate pathway late in its course it was necessary to demonstrate that the two stimulating electrodes were activating two different pathways to the cortex. The following points support this view:

1). Firstly, the responses to CL and OR_1 stimulation differed from one another. Figure 4 shows a representative example of the reponses of a striate neuron (a C cell) to stimulation at OR_1 and CL and, in this case, the responses differ both in their evoked potentials and in the spread of jitter of repeated responses. For different cells, Fig 5 makes a comparison of the variation in response jitter after stimulation at CL and OR_1. In each penetration, there were many cells where the response patterns were too different to be consistent with single pathway stimulation.

Fig.4: Oscilloscope tracings from a striate neuron showing the cluster of responses following stimulation repeated 1Ø times at OR_1 (upper trace) and CL (lower trace). Although jitter is present in both responses it is more marked in the CL trace. Note also that the slow or evoked potential differ in the two traces. Scale bar: Vertical, 1mv; horizontal, 2ms

Response to stimulation

At OR_1



At CL

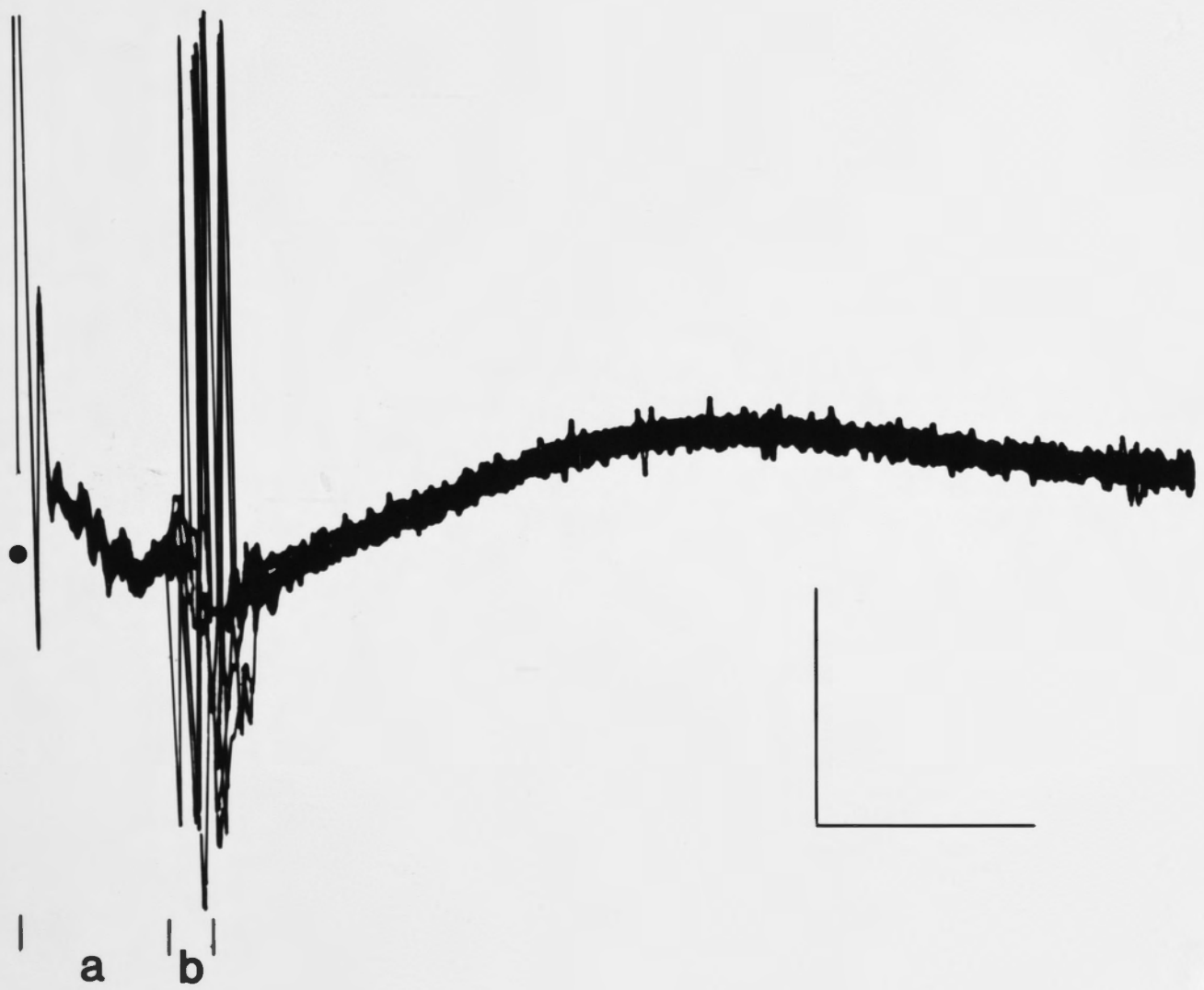
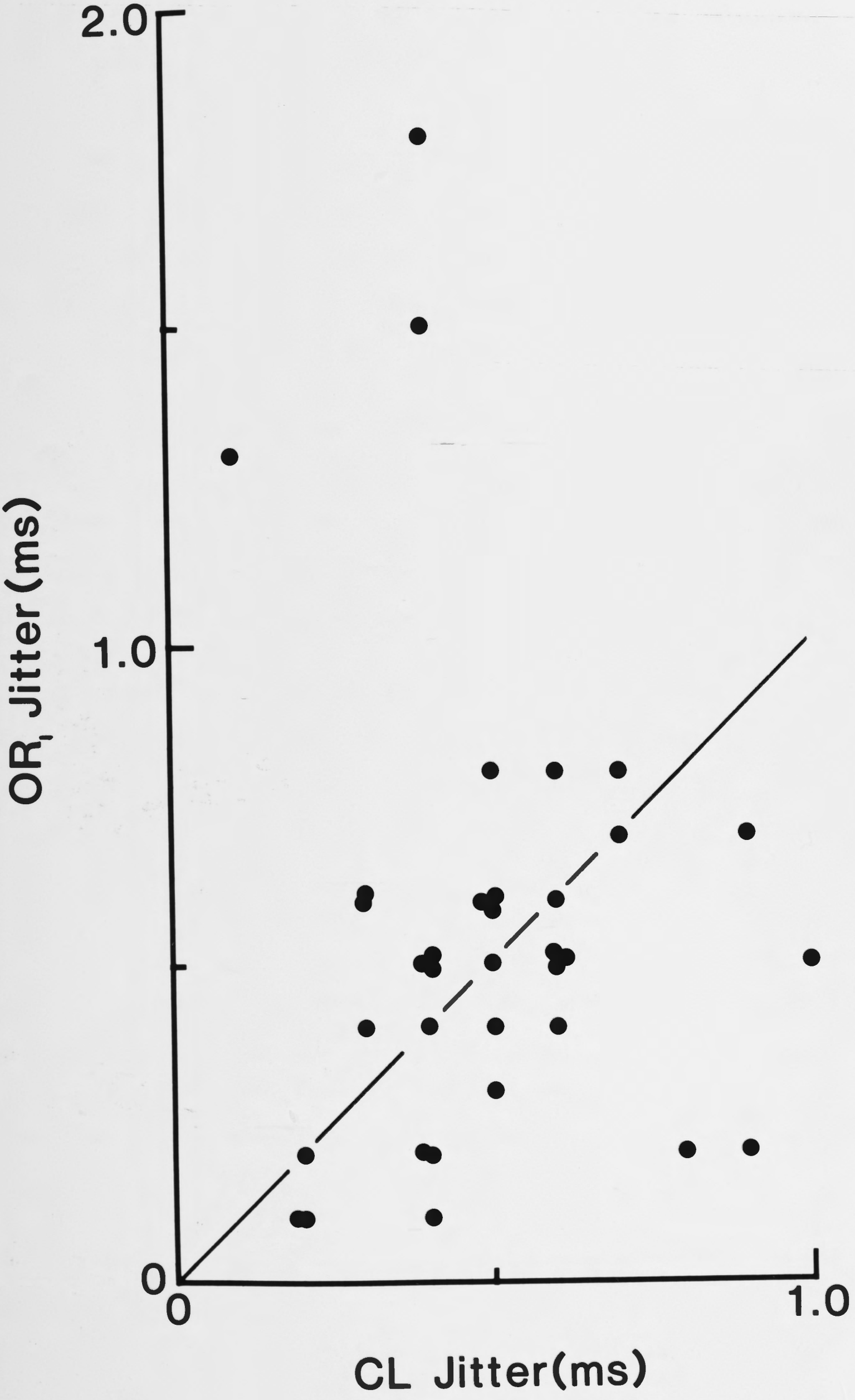


Fig. 5: A comparison of the spread (or jitter) in responses in individual striate neurons to repeated stimulation (see FIg. 4) at OR_1 (LGN) and CL (claustrum). Differences in the level of jitter from the two stimulating sites suggests that separate paths carry the signals from OR_1 and CL to the striate cortex.


2.0
1.0
0
0
1.0
OR, Jitter (ms)
CL Jitter(ms)

2). Secondly, the ratio of CL to OR_1 latencies was not consistently above or below 1. In fact, in a single penetration, the ratio of CL/OR1 latencies varied from being above 1 in one cell to below 1 in the next. Also, from the full sample in Fig 6, it may be seen, that despite the greater apparent distance to CL, about half of the sample (34/72) had an OR_1 latency that was either longer than (22 cells) or equal to (12 cells) the CL latency.

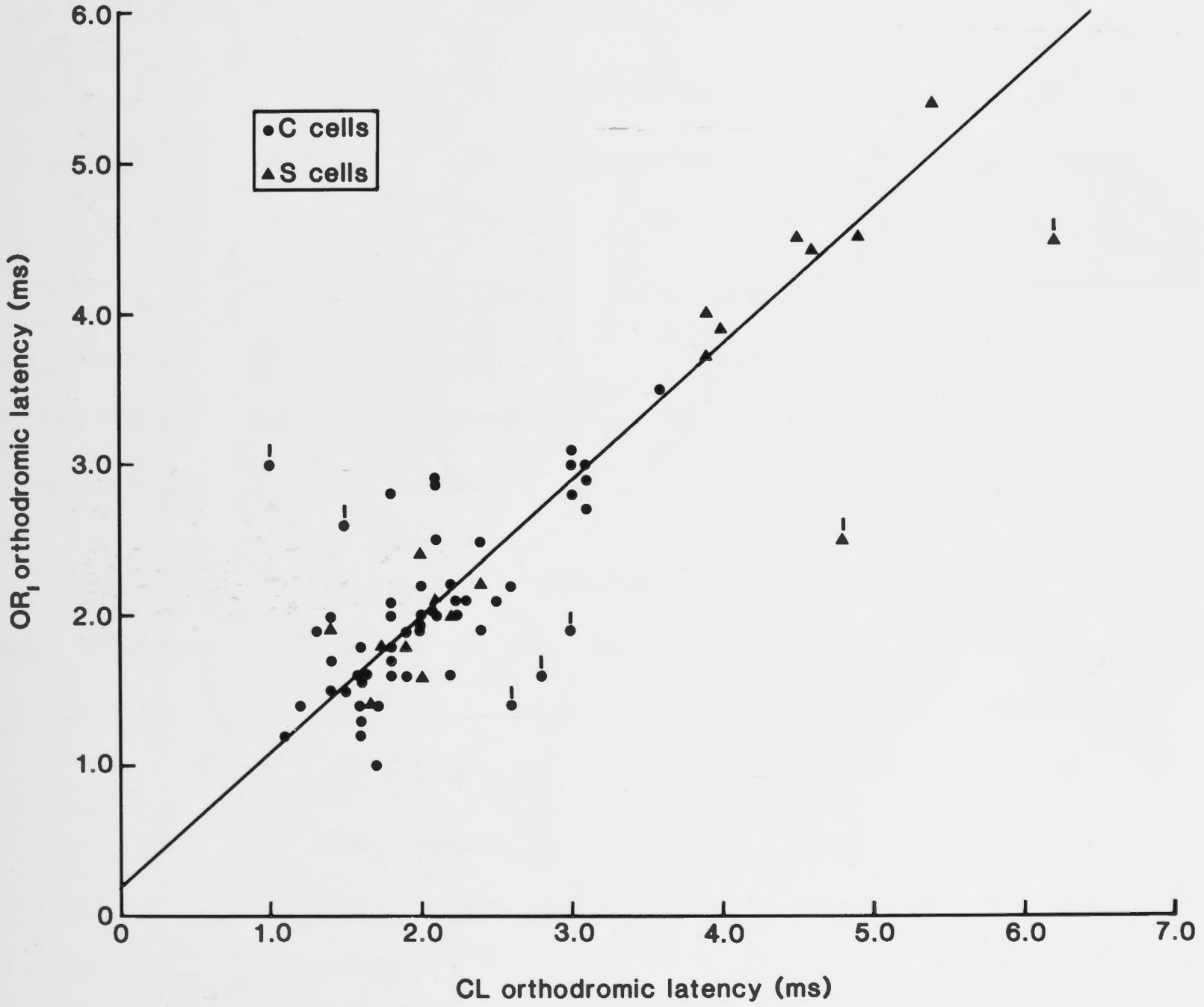
3). In addition, many more cells were recorded with antidromic responses (13 compared to 1) from the OR_1 electrode than from the CL electrode. Often in these instances there was an orthodromic or, in otherwords, a dissimilar drive from the CL electrode.

4). Towards the end of some of the experiments, tests were made on the effectiveness of the local anaesthetic, lidocaine, to deactivate the claustrum. In the eight striate neurons tested with a inactivated claustrum the drive from CL was lost while that from OR_1 remained unmodified.

5). On topographical grounds, the claustral electrode, in particular, was always inserted well into the nucleus, being 2-3 mm removed from its caudal boundary and previous experience (Bullier et al., in preparation) suggests that activation over distances greater than 2mm is unlikely.

6). Finally, experiments reported in Chapter 4, using anterograde transport of WGA-HRP, have shown that the claustro-striate pathway passes well above (more than 3mm)

Fig. 6: A comparison of the orthodromic or transynaptic response latencies in individual striate neurons following stimulation at OR_1 (LGN) and CL (claustrum). The regression line, considered to be more representative of the majority of cells, is derived from a sample that excludes the points marked with a vertical tag. The highly significant coefficient of correlation for this abridged sample reflects a pairing of similar inputs from the two pathways.


6.0
5.0
4.0
3.0
2.0
1.0
0
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
•C cells
▲S cells
OR1 orthodromic latency (ms)
CL orthodromic latency (ms)

the LGN (Boyapati et al., 1985) and that there is little likelihood of its activation from OR_1 (Bullier and Henry, 1979a).

In view of all this evidence, it was decided that the OR_1 and CL were activating separate pathways running to the cortex.

A comparison of signal conduction from OR_1 and CL

After confirming that the CL and OR_1 electrodes were stimulating separate pathways to the cortex, a comparison was made of conduction characterstics in the two paths. This is illustrated in Fig 6 where, for each cell, the CL latency is plotted against the OR_1 latency, and the points are found cluster around the line of zero difference between the two latencies.

In the statistical analysis the co-efficient of correlation was determined for two samples- one for all available cells with both OR_1 and CL latency measurements (all points in FIg 6) and the other where the 7 cells, represented by the tagged symbols, were removed from the sample. For the cells with tagged symbols the two latencies, OR_1 and CL, differed by more than 1.Øms and in these cases the two paths either had markedly divergent conduction characteristics or possibly inadvertently, a first response in one path was being compared with the second order response in the other (Bullier and Henry, 1979a). Whatever the reason for the divergence in their latencies, these were judged to lack a correlation in their

CL and OR_1 pathways and were removed from the sample to avoid distorting the findings for cells with a potential relationship. This step resulted in the subtraction of 7 of the 71 cells and produced a more representative regression line in Fig 6 without modifying the interpretation of the statistics. Thus for both samples there was a significant correlation as follows: for the full sample of 72 cells r: Ø.85; d.f.: 7Ø; p <Ø.ØØ1 and for the amended sample of 65, r: Ø.94; d.f: 63; p< Ø.ØØ1.

Recipient cell types

Encountered cells with a claustral drive were classified into C and S families (Henry, 1977). There was little need to further subdivide into C and B categories (Henry et al., 1983) as only two B cells were encountered with a claustral drive. Since subclasses in the S family are difficult to recognize from their responses to visual stimuli (Mustari et al., 1982) it was also treated as a single group, although, in the analysis of the results, a distinction was drawn between S cells with large and small receptive fields. From the population of classified cells (9Ø%) with a claustral drive, 68% were found to be C cells, and 26% were S cells (see Fig. 4). The remainder of the sample were either B cells or had non-oriented receptive fields.

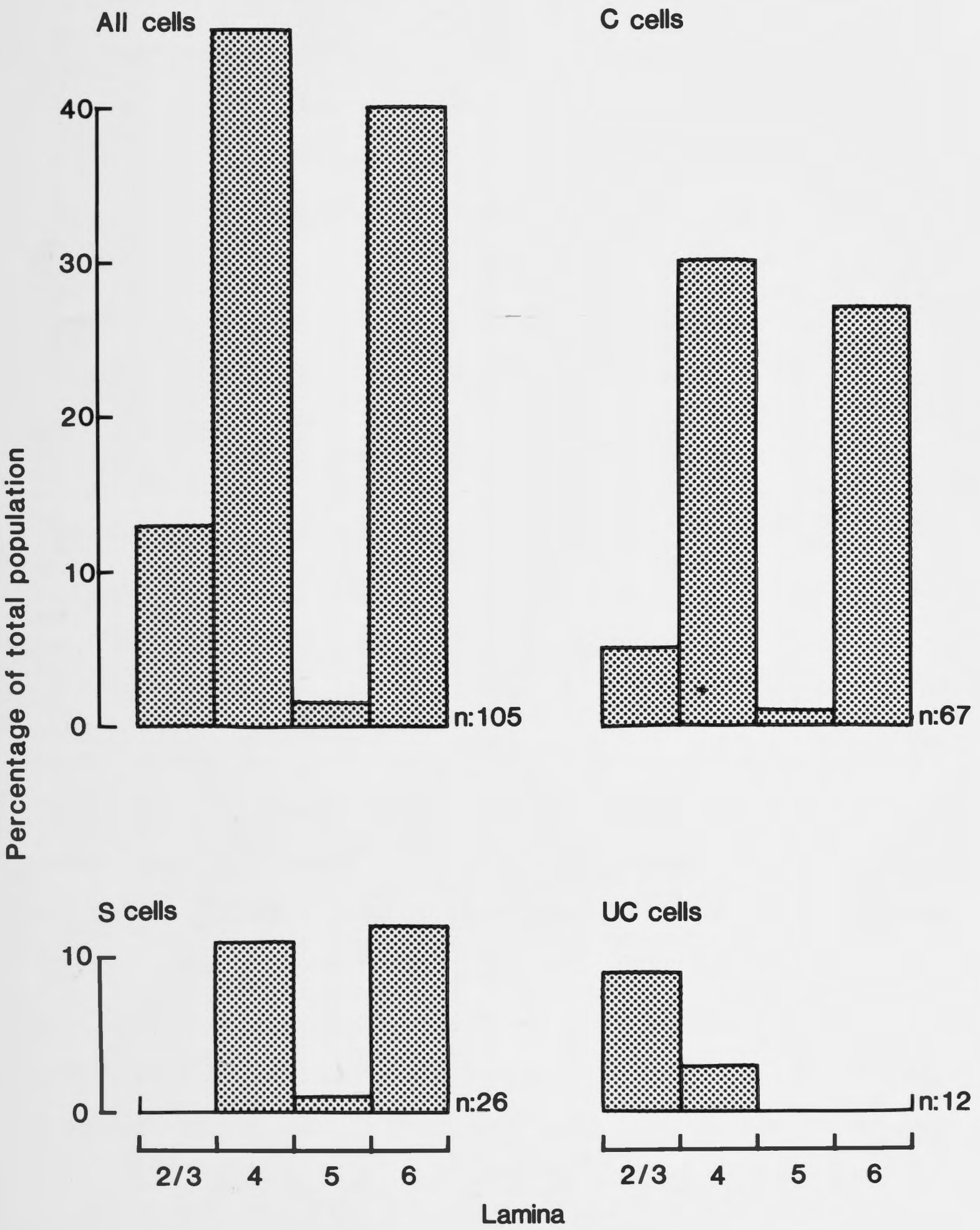
Laminar distribution of recipient striate neurons

An ordered laminar arrangement of claustral terminals in the striate cortex was first described in the tree shrew (Carey et al., 1979; 198Ø) Anatomical evidence, based largely on the transport of radioactive amino acids (LeVay and Sherk, 1981a) indicates that in the cat the claustro-striate pathway terminates principally in laminae 4 and 6. The distribution of claustral-driven cells in this study conformed quite well with this pattern so that 44% of the sample were encountered in lamina 4 and 4Ø% in lamina 6 (see Fig. 7). These figures should not be taken as an accurate indication of laminar distribution since there was not an equal probability of encountering cells in each lamina. It appears to be significant, however, that only 2 claustrally driven cells were encountered in lamina 5 despite the fact that all 11 electrode penetrations passed through this lamina.

With regard to the laminar distribution of cell types with a claustral drive, Fig. 7 shows that both C and S cells were encountered in laminae 4 and 6. Approximately equal proportions of each class were found in these two laminae.

After discovering the concentration of claustral-driven cells in laminae 4 and 6, the electrode was directed to these laminae so that the distribution was biased to the extent that there was a different level of sampling in the various laminae. The distortion is probably not great, however, since the path taken by most of the recording

Fig. 7: Laminar distributions of encountered claustral driven cells in the total sample and belonging to C, S and UC (unclassified) categories. All percentages are expressed in terms of the total sample.


All cells
C cells
Percentage of total population
40
30
20
10
0
n:105
n:67
S cells
UC cells
10
0
n:26
n:12
2/3
4
5
6
2/3
4
5
6
Lamina

electrodes went through both laminae 4 and 6 and of necessity spent some time in laminae 2, 3 and 5. The reconstruction of an electrode track in Fig. 8 shows a typical encounter profile. The geometric symbols represent cells with a claustral-drive; the triangles stand for S cells, the filled circles for C cells, and the open circle for a B cell, the bars stand for cells with out a claustral drive. Too much significance should not be placed on the relative proportions of encountered cells but it was interesting to note that almost half of the recorded cells in laminae 4 and 6 received an input from the claustrum.

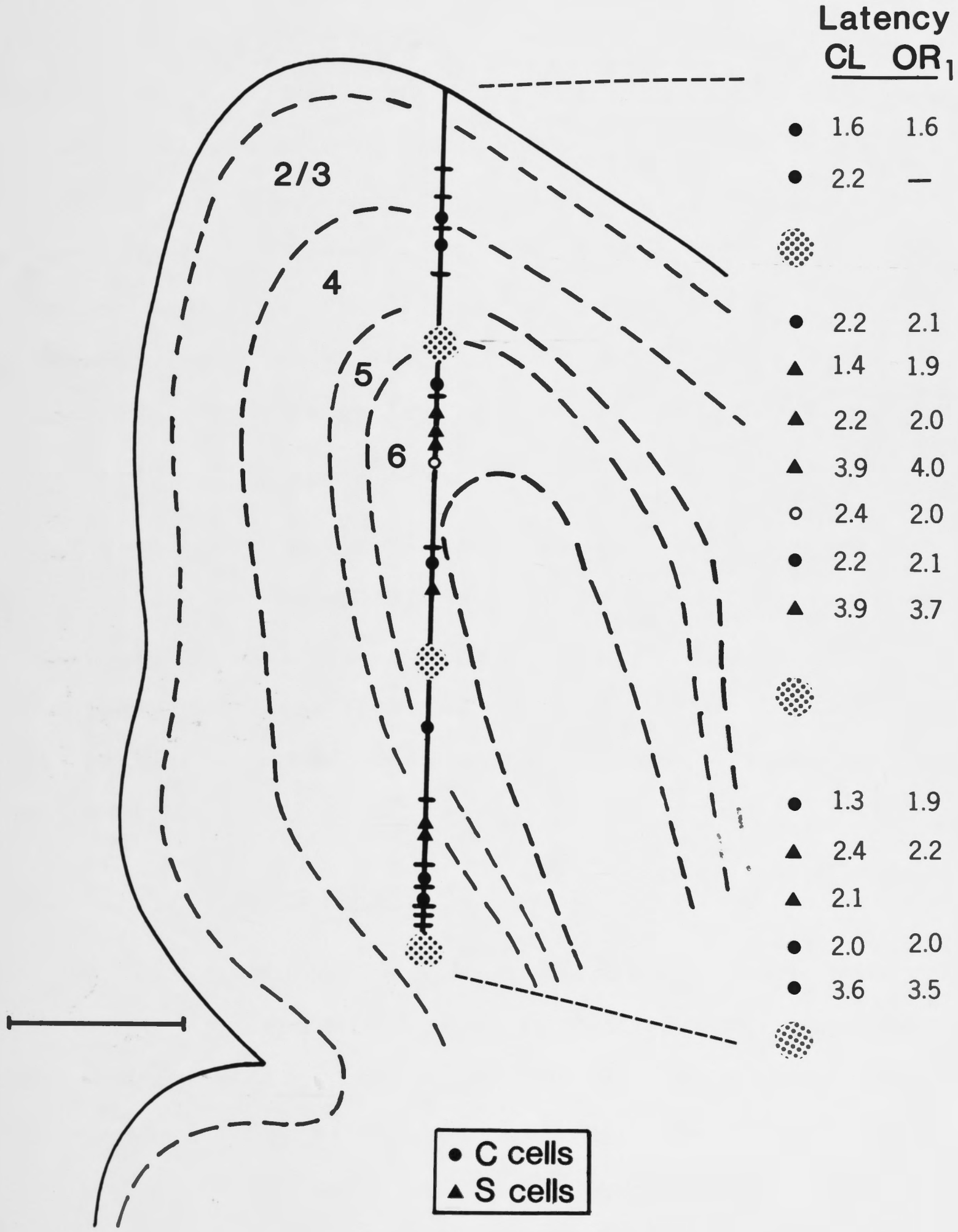
II VISUAL RESPONSE PROPERTIES OF CLAUSTRAL-DRIVEN CELLS

Following classification with hand held stimuli, about a quarter of the sample was further examined by subjecting their responses to a quantitative evaluation.

(a) Receptive field dimension

The primary dimension of the receptive field, the distance from the front to the back border, was measured from the extent of the peak in average response histograms to narrow moving bars. This measurement was made for 18 cells: 1Ø C cells, 7 S cells and one B cell. For the C and B cells the measurement covered the entire discharge region while with S cell it was restricted to the extent of the dominant subregion (Mustari et al., 1982). Since the primary dimension can be accurately measured with hand-held stimuli, estimates made in this fashion on 12 S cells were added to those derived from average response histograms.

Fig. 8: Typical path taken by the recording electrode through the laminae of the striate cortex. The stippled areas represent electrolytic lesions (5μs for 5s) and the geometric symbols, represent claustral-driven cells, - the filled circles stand for C cells, the open circle, for a B cell and the triangle, for S cells- the horizontal bars represent encountered cells with no detected claustral-drive. Scale bar, 1mm.


Latency
CL OR1
1.6 1.6
2.2 —
2.2 2.1
1.4 1.9
2.2 2.0
3.9 4.0
2.4 2.0
2.2 2.1
3.9 3.7
1.3 1.9
2.4 2.2
2.1
2.0 2.0
3.6 3.5
2/3
4
5
6
• C cells
▲ S cells

From the measurements of the primary dimension (see Table 1) it was apparent that the receptive fields of claustral driven cells had dimensions similar to those reported previously for the general population of S (Mustari et al., 1982) and C (Henry et al., 1983) cells. In the S cells, however, there was an indication that cells belonging to different CL latency groups had different receptive field dimensions. For those with a fast input from the claustrum (CL latency 2.5ms) the mean primary dimension was Ø.45° (n: 13; range, Ø.34° to Ø.76°); for those with a slow input (CL latency 3.Øms) the mean was Ø.71° (n: 7; range, Ø.55° to Ø.88°).

The lateral extent of the receptive field was also measured for claustral-driven cells. This measurement was derived from the length-response curves that were used also to estimate end-zone inhibition in the receptive field and the presentation of data on the lateral dimension is delayed until section (e) below.

(b) Velocity tuning curves

Velocity tuning curves, recorded from average response histograms prepared by interleaving the responses to narrow bars moving at different velocities, were obtained from 6 S cells and 14 C cells. The findings were measured in terms of optimal stimulus velocity and the fastest effective stimulus and the resulting statistics, presented in Table 1, were similar to those for the general population (LeVay and Sherk, 1981b, Mustari et al., 1982).

TABLE 1

Response properties of claustral-driven striate neurons

CELL TYPE		RECEPTIVE FIELD DIMENSIONS (deg.)						END ZONE INHIBITION (%)		
		Primary			Lateral					
	Latency	Mean	Range	n	Mean	Range	n	Mean	Range	n
	(ms)									
S	>3.0	0.4	0.3 - 0.7	13	0.9	0.5 - 2.6	3	12.0	0 - 36	3
	<2.5	0.7	0.6 - 0.9	6	3.9	1.8 - 6.2	4	18.8	0 - 33	4
C		2.7	1.8 - 3.8	10	2.3	0.3 - 5.4	10	19.3	0 - 39	10

CELL TYPE	ORIENTATION SPECIFICITY (deg.)			VELOCIITY TUNING CURVE (deg./s)					
				Optimal Value			Cut Off Value		
	Mean	Range	n	Mean	Range	n	Mean	Range	n
S	17.6	14.1 - 19.1	6	1.9	0.8 - 3.0	6	8.3	4.0 - 15.0	6
C	27.4	22.5 - 30.5	6	4.7	1.5 - 10.0	14	14.3	7.0 - 18.8	14

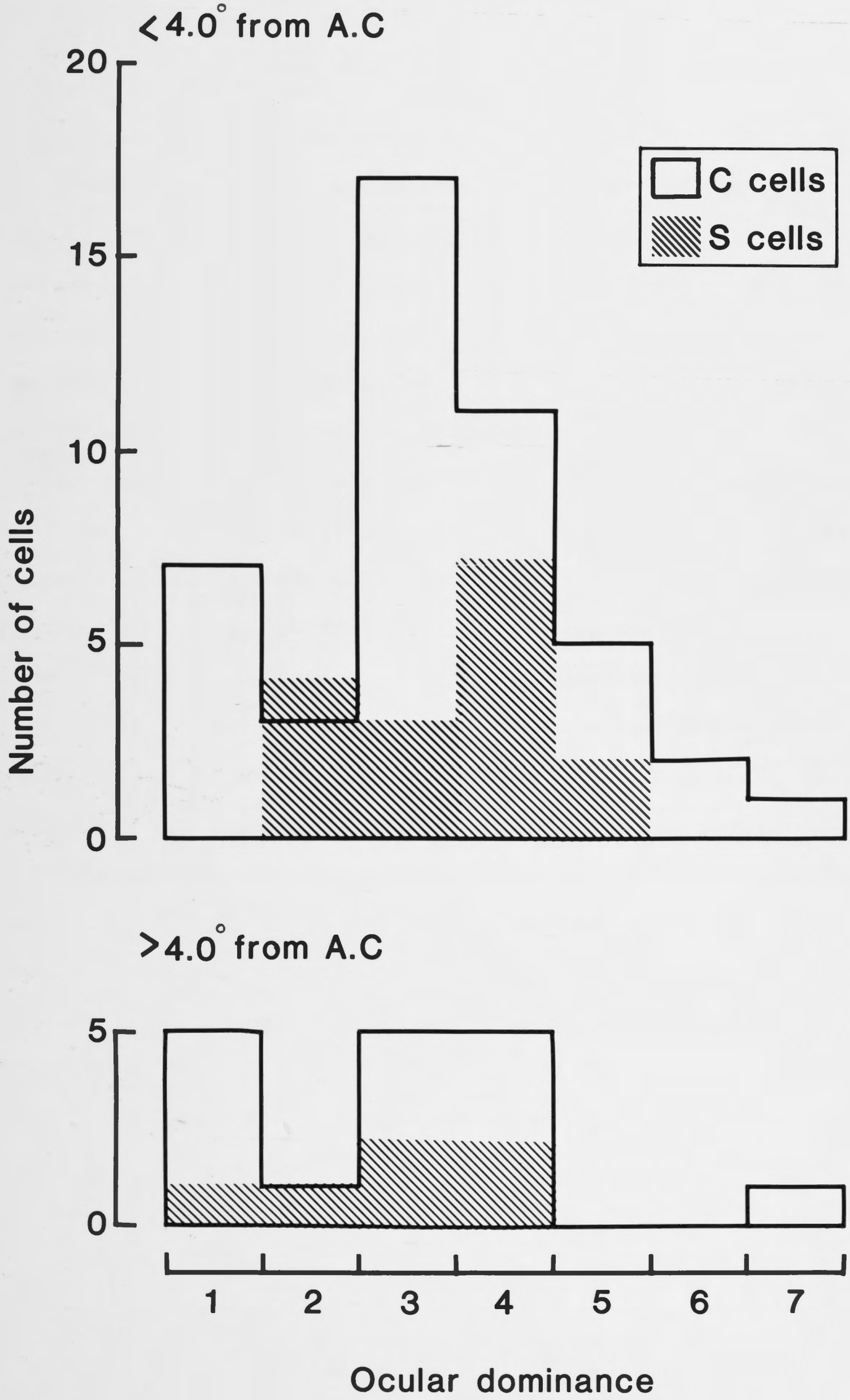
In summary, C cells had a slightly higher cut off and optimal velocity than S cells but, in both cases, no cell was found where the optimal value exceeded 1Ø°/s or the cut off was greater than 2Ø°/s. These are similar to the results obtained in the general population but contrast with the generally higher values found for claustral cells (Sherk and LeVay, 1981). Nothing in the shape or position of the velocity tuning curves in the sample, however, suggested that claustral-driven cells were members of the distinctive sub-group of striate neurons.

(c) Orientation tuning curves

Orientation tuning curves were prepared from average response histograms that were constructed by changing the orientation of the stimulus with each successive stimulus presentation. In all instances the stimulus was a narrow long bar (longer than 1Ø°) moving across the receptive field. From the resulting orientation-response curves the acuteness of the tuning curve (half width at half height) was estimated for 6 S cells and 6 C cells and presented in Table 1.

The acuteness of tuning in the claustral-driven cells was almost identical to that of the general population (Henry et al., 1974). Thus, the mean for S cells was 17.6° (compared to 17.Ø°) while that for C cells was 27.4° (compared to 27.5°) and again there was no obvious distinction associated with a claustral link.

Fig. 9: Ocular dominance histograms for claustral-driven striate neurons with receptive fields within and beyond 4^{o} of the middle of the area centralis. The ocular dominance rating devised by Hubel and Wiesel (1962) has exclusively monocular drive at 1 (contra-lateral) and 7 (ipsi-lateral) and an equivalent drive from each eye at 4.


<4.0° from A.C
20
15
10
5
0
Number of cells
C cells
S cells
>4.0° from A.C
5
0
1
2
3
4
5
6
7
Ocular dominance

(d) Ocular dominance

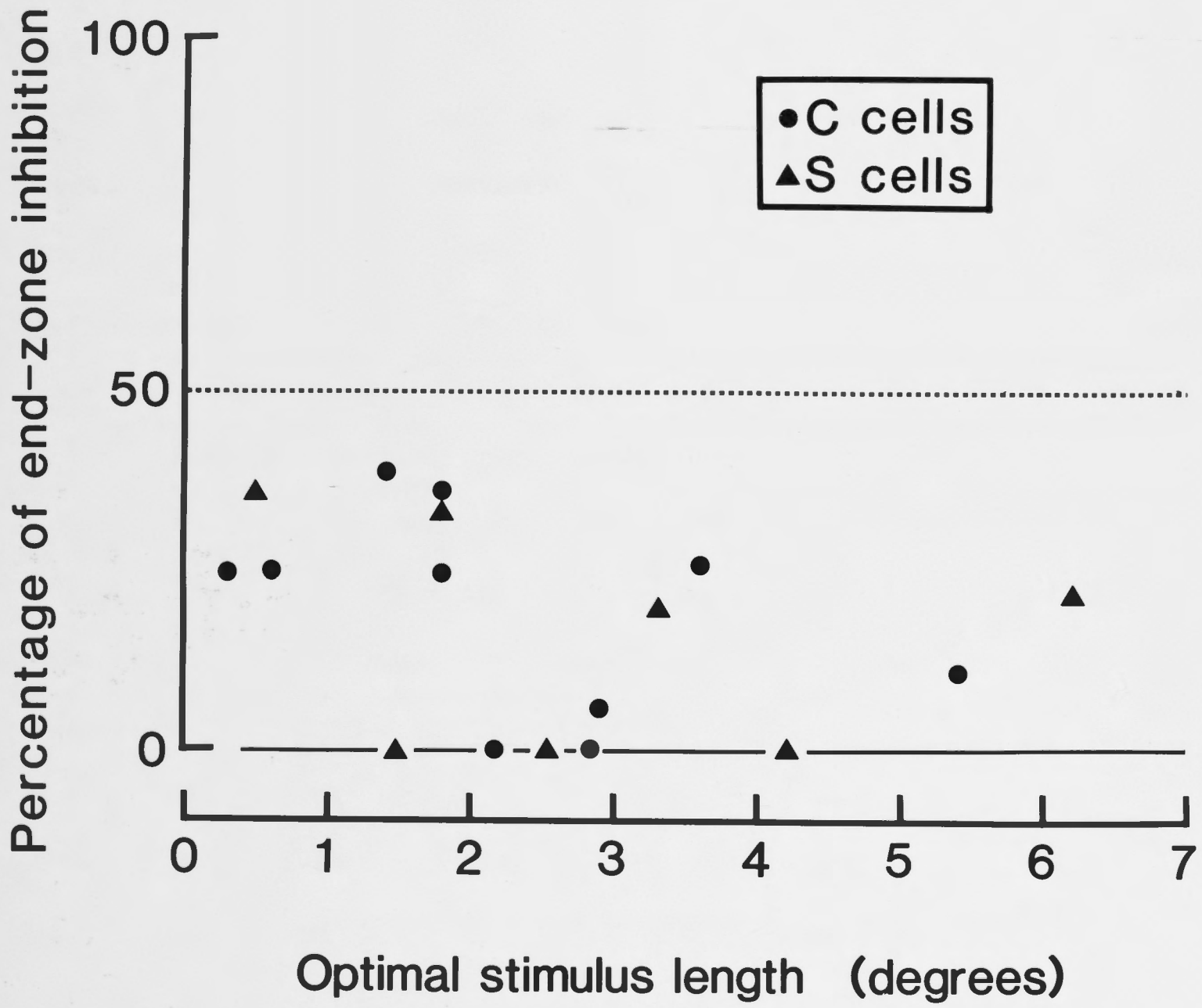
The effectiveness of either eye in driving claustral-linked striate neurons was recorded with hand held stimuli according to the criteria of Hubel and Wiesel (1962). Taken as a whole, the sample displayed a distribution of ocular dominance similar to that originally presented by Hubel and Wiesel (1962) and showed a preponderance of cells with a binocular influence. Recently, however, it has been suggested that this pattern is confined to cells with eccentric receptive fields and that the majority of cells with central receptive fields (within 4.0^{o} of the mid point of the area centralis) are not binocularly influenced (Albus, 1975). To examine this aspect of the distribution, the ocular dominance histograms in Fig. 9 were prepared for S and C cells which were subdivided into groups with receptive fields inside and outside the 4^{o} line circling the area centralis. From Fig 9 it is apparent that most claustrally driven cells with centrally located receptive fields received a similar input from both eyes. There were some signs in the C cell population that representatives with a monocular drive were more frequently influenced by the contra- than the ipsi-lateral eye but the most common occurence was for cells to belong to groups 3 or 4 irrespective of the eccentricity of their receptive fields.

(e) Optimal stimulus length and end-zone inhibition

End-zone inhibition, the property that promotes the cell's preference for foreshortened stimuli, was measured from average response histograms constructed together for a range of stimulus lengths. Starting at a length of Ø.1° (and keeping a width of Ø.2°) the stimulating bar was lengthened in steps of Ø.5° until there was no noticeable increment in successive responses. Optimal stimulus lengths were determined from length-response curves and the intensity of end-zone inhibition was assessed from the extent to which to response to optimal length was diminished by further lengthing. This method was applied in earlier studies to measure the incidence of end-zone inhibition for both S and C cells in the general popoulation of striate neurons (Henry et al., 1983; Mustari et al., 1982). The measurements taken from length-response curves from 17 claustral-driven striate neurons are shown in Fig. 1Ø. End-zone inhibition is expressed as the percentage of response reduction that occurred when the stimulus was lengthened beyond its optimal value. For each cell the optimal length is plotted on the abscissa.

The results from these experiments confirmed an observation made with hand held stimuli that, in the sample of claustral-driven cells, there were very few with strong or even moderate end-zone inhibition. Overall the end-zone inhibition of the 17 cells in Fig. 1Ø averaged 16.6% and the most intense inhibition was 39%. It was thought that low level of end-zone inhibition may have been associated

Fig. 1Ø: Scattergram showing the strength of end-zone inhibition plotted against the length of bar that produced the maximum excitatory response. End-zone inhibition, expressed as a percentage of the optimal response, was quantitatively determined and found to be weak (less than 4Ø%) or absent in the tested claustral-driven cells. Note also that the optimal stimulus length did not exceed 6.7° in any of the tested cells.


Percentage of end-zone inhibition
100
50
0
•C cells
▲S cells
0
1
2
3
4
5
6
7
Optimal stimulus length (degrees)

with the high proportion of C cells but, as Table 1 shows, claustral-driven S cells also had weaker end-zone inhibition than commonly found in the general population (Mustari et al., 1982). The sample appeared to be distinctive, in that end-zone inhibition, if present at all, exerted only a weak influence on the cell's response.

Another feature, that once again may arise from the large representation of C cells, is to be seen in the distribution of optimal stimulus lengths (or lateral receptive field dimensions) recorded in Fig. 1Ø. It has been shown (Sherk and LeVay, 1983) that claustral cells themselves have a preference for long stimuli (up to and beyond 3Ø^{o} in length) but this predisposition did not seem to be reflected in the responses of striate neurons in the sample. From Fig. 1Ø, the longest optimal stimulus was 6.2^{o} and for the majority of cells it was less than 3^{o}, a value similar to that observed previously in the general population of cells in lamina 4 (Henry et al., 1983; Mustari et al., 1982). The optimal stimulus length, recorded as the lateral dimension of the receptive field in Table 1, averaged 2.7^{o} for the 17 cells in Fig. 1Ø.

(f) Direction selectivity

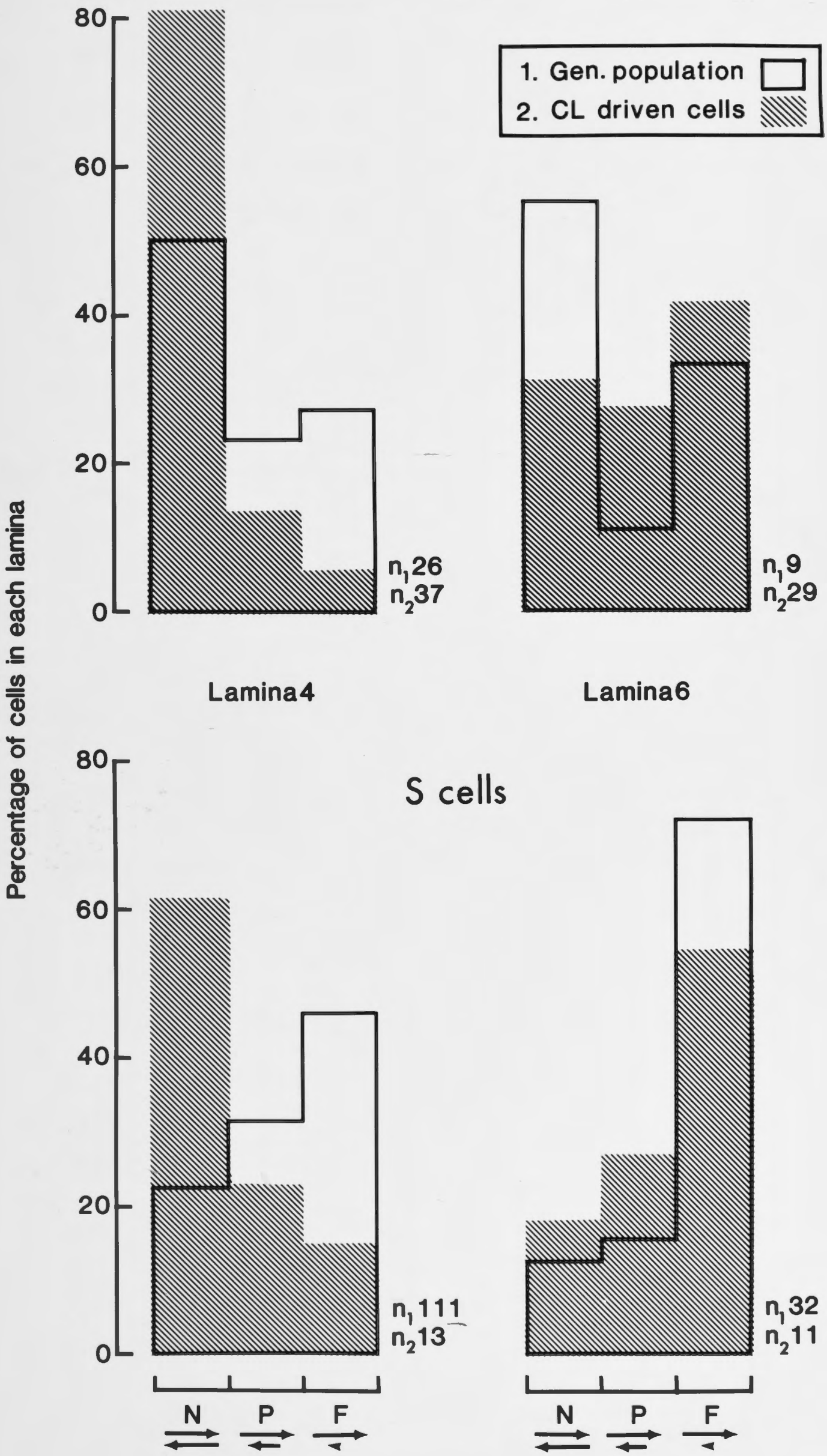
The results for direction selectivity were based on both the responses to hand held stimuli and computerized evaluations that placed a cell in one of three categories to indicate that it was not (N), partially (P) or fully (F) direction selective.

The direction selectivity of cells with a claustral drive is shown in Fig. 11 where it is compared with previously unpublished results taken from experiments in which direction selectivity was measured along with other aspects of the striate neuron's response (Bullier et al., 1982; Henry et al., 1983; Mustari et al., 1982). From the results in Fig. 11, there appeared to be a distinction in the degree of direction selectivity shown by cells in different laminae. Thus, most of these cells in lamina 6 were direction selective while only a minority were direction selective in lamina 4. This was the case, irrespective of the cell type although direction selectivity was slightly less dominant in the C than the S cells of lamina 6.

By contrast, directional differences in the general population was more a feature of cell type than laminar disposition. A higher proportion of S than C cells, whatever their lamina, displayed a strong direction dependence. Directional and non-directional dependent C cells occurred in approximately equal proportions in both laminae.

The most striking distinction in the sample of claustral-driven cells, therefore, was that those in lamina 4, belonging to either the S or C families, were less direction selective than their counterparts in the general population. This distinction was more obvious for S cells because the S cells of the general population were predominantly direction selective. Statistically, the

Fig. 11: A comparison of direction selectivity in striate neurons with a claustral-drive against those in the general population. Shaded histograms represent cells with claustral-drive; open histogram, the general population derived from unpublished data obtained in earlier studies on the striate cortex (Bullier and Henry, 1979 a&b; Mustari et al., 1982). Direction selectivity was measured from the relationship $[R_P/(R_P+R_{NP})]x100$ and then grouped as follows: N: none (50-65); P: partial (65-85) and F: full (85-100).


C cells
1. Gen. population
2. CL driven cells
80
60
40
20
0
n1 26
n2 37
n1 9
n2 29
Lamina4
Lamina6
Percentage of cells in each lamina
S cells
80
60
40
20
0
n1 111
n2 13
n1 32
n2 11
N
P
F
N
P
F
Direction selectivity

difference between claustral-driven cells and those of the general population resulted in the following X^2 values:

Lamina 4: S cells = 88.55; C cells = 39.82

Lamina 6: S cells = 15.51; C cells = 35.19

(in each case df = 2 and p. < ØØ1)

NOTES ON INTERPRETATION OF RESULTS

Problems in characterizing the claustral drive

The authenticity of orthodromic drives in the striate cortex has been questioned on the grounds that the signal may pass back along a cortical efferent into a intra-cortical collateral, which terminates on the recorded cell. For two reasons this seems unlikely in the claustro-striate pathway. Firstly, as mentioned earlier, radioactive D-aspartate filled the collaterals in lamina 4 of retrogradely infiltrated striate neurons following injection into the LGN but not when the injection was made into the claustrum (Baughman and Gilbert, 1980; LeVay and Sherk, 1981a; see also Katz, 1984). The rarity with which cells with an antidromic drive were encountered also would suggest that trans-synaptic drive through the collateral of a cortical efferent would likewise be a rare event. There are reasons to conclude, therefore, that the orthodromic responses recorded in striate cells arise from signals that pass along the axons of claustral cells.

Even with a true orthodromic response, however, there are difficulties in characterizing afferents in the claustro-striate pathway. For example, it is more of a problem to assess the ordinal position of a striate neuron receiving from the claustrum than from the LGN. In earlier studies on the geniculo-striate pathway a stimulating electrode was placed high in the optic radiations close to

the point of termination of the incoming axon (the OR2 electrode of Bullier and Henry, 1979a). For this electrode, the latency contribution due to axonal conduction was reduced in favour of that arising from synaptic delays. In the present experiments on claustral-drive, however, OR_2 stimulation was not used since it would have been difficult to distinguish afferents of claustral origin from those coming from the LGN. As a result, and the determination of ordinal position was based only on the length of the OR_1 latency.

A second difficulty accompanies the measurement conduction times in axons coming from the claustrum. In the geniculo-striate pathway, the $OX\text{-}OR_1$ latency difference (where OX stands for the optic chiasm as a stimulating site) provides an estimate of the conduction time in an isolated length of the afferent path (Bullier and Henry, 1979b). A similar portion of the claustro-striate pathway was not accessible and stream identification depended only on differences observed in the CL latency. Without knowledge of the number of synaptic crossings contributing to the CL latency there was uncertainity in the time for axonal conduction and, therefore, the nature of the functional stream. However, many of the problems associated with stream identification could be avoided because so many cells had the short CL latency consistent with a fast monosynaptic input.

Another problem in the interpretation of the results is the question of whether the distribution of cell types with

a CL drive is biased because inhibitory pathways suppress the response in certain cell types. To some extent such an effect has been observed in the past and it was found that C cells, which usually display a low level of inhibition in their receptive fields, are driven more consistently from OR_1 than S cells. However, this does not mean that S cells, with their more obvious inhibitory receptive field regions, are not driven in large numbers but rather that their responses to repeated stimulation are not as well duplicated as in C cells. Allowing for this aspect of the response to electrical stimulation, no predominance of C cells was found to be driven from OR_1 and, therefore, the high incidence of C cells with a claustral drive appears to be a genuine trend.

DISCUSSION

Conduction streams in the claustro-striate pathway

The high proportion of axons with relatively fast conduction times proved to be an outstanding feature of the claustro-striate pathway. The possibility that these axons go to make up a fast stream seems all the more likely when the situation in the claustro-striate pathway is contrasted with that in other pathways entering the striate cortex. Thus, the 69% of cells, with a CL latency of less than 2.5ms differs markedly from the 16% and Ø% of the striate neurons driven with similar latencies from Areas 18 and 19 respectively (Bullier et al., in preparation). Despite their shorter conducting distance, samples of cells driven from Areas 18 and 19, taken as a whole, had much longer latencies (means: 6.1ms and 1Ø.4ms, respectively) than found in the claustro-striate pathway (overall mean: 2.6ms). On the other hand, a fast conducting stream distinguishes the geniculo-striate pathway where the distribution of OR_1 latencies for striate neurons is almost identical to that for CL latencies in claustral-driven cells (Bullier and Henry, 1979a).

In an earlier study into the geniculo-striate pathway (Bullier and Henry, 1979b), it was possible, using another latency measurement, to subdivide the cells represented in the first peak of the OR_1 latency distribution. These cells, thought to be monosynaptically driven from the LGN, could be separated, according to the value of their $OX\text{-}OR_1$ latency difference, into groups with fast and slowly

conducting axons arising from Y and X cells, respectively. Although, a measurement equivalent to OX-OR_1 was not available in the present study, there were other indications that fast conducting axons were providing the input to cells in the fast CL latency peak.

The first indication that the cells with a short CL latency belonged to one type of input came with the finding that so many (55/64) were C cells, a group known to receive a fast or Y input from the geniculo-striate pathway (Bullier and Henry, 1979b; Henry et al 1983). This raised the possibility that a input of similar character converged on these cells from both the LGN and claustrum. The comparison of CL and OR_1 latencies, considered valid in light of the apparent independence of the responses to stimulation at the two sites, revealed a strong correlation in signal conduction times in the converging pathways. This relationship held quite strictly across the sample to the extent that 58 of the 72 cells differed by less than Ø.5ms in their two latencies.

The latency measurements seem to suggest, therefore, that the fast stream is an important component of the claustro-striate pathway, that its major target is the C cell of laminae 4 and 6 and that for most of these cells (only 11 of the 6Ø cells in the first peak failed to be driven from OR_1) there is an equivalent drive from OR_1. The convergence onto a single cell of afferents coming from the claustrum and the LGN contrasts with the arrangement for striate neurons in lamina 6 that feed the reciprocal

pathways. Here, the tracer experiments indicate that different cells project to each of the two subcortical nuclei (LeVay and Sherk, 1981a).

The S cells represented in the first peak of the CL latencies also are likely to be driven by a fast pathway from the claustrum and the C cells with CL latencies clustering aroud 3.Øms could also receive from the fast pathway (see Figs. 2 and 6). These cells, which make up a small second peak in the distribution, may be second-order neurons. The average time taken to travel from a primary receiving cell to the next cell in the sequence is 1.1ms for the cat striate cortex (Bullier and Henry, 1979a) and 3.Øms (1.9+1.1ms) would then be the expected latency for a second order neuron.

An examination of the functional responses of the S cells in the first peak of CL latencies, shows that their receptive field dimensions were the same as those of S cells with a fast or Y input from the LGN. Thus, like the C cells of the first peak these S cells also appeared to receive a Y stream input from the LGN and a similar fast input from the claustrum.

The design of the claustro-stiate pathway, however, may not be exclusively dedicated to the fast conduction of signals. Tsumoto and Suda (1982) found cells with very long latency CL drive (13 to 3Øms) and in the present study there is a group of S cells with latencies ranging from 3.Øms to 6.8ms. These cells may well receive signals from a slow stream but their input could also be delayed by the

presence of two or more synapses. Such polysynaptic inputs are difficult to activate with electrical stimulation (Bullier and Henry, 1979a), however, and it is more likely that the input comes from a slow stream. As with cells presumed to receive an input from the fast stream, these long latency S cells were also activated by a path of similar character running from the LGN.

Reviewing the composition of the claustro-striate pathway, as revealed from electrical stimulation, the major component appears to be a fast stream that terminates principally on C cells, and to a lesser extent on S cells, both of which reside in laminae 4 and 6. A secondary slow stream, also appears to be present and to terminate on a separate group of S cells that occur mainly in lamina 4. A general rule for striate neurons receiving from the claustrum and the LGN, may be that "like" axons from the two pathways converge on to a single cell.

The claustral loop

The course taken by signals passing through the striate cortex and then through the claustral loop starts with the pyramidal cells of lamina 6. With their potential to be mono-synaptically driven from the LGN, signals could pass quickly through these cells and set out on their way to the claustrum. The claustral-projecting pyramids may be exceptional in that, lacking collaterals running into lamina 4, their signals are destined to arrive in lamina 4 only after travelling via the claustrum. On reaching the

claustrum the visual input comes under the influence of auditory and somato-sensory signals and the role of the claustrum would be to act as an interchange station where signals from the various visual cortices relate with those from the two other modalities. The return from the claustrum could take two forms -a feed back going to lamina 6 and the continuation of the loop that re-enters in lamina 4. This second arm of the loop is a fast conducting pathway and, if the outward arm were similar, then the overall journey through the loop would be completed within a much shorter interval than for similar loops through Areas 18 and 19 (Bullier et al., in preparation).

The claustral contribution to the responses of striate neurons

Earlier studies have attributed end-stopping in the responses of cells in laminae 2/3 and 4 as the most pronounced influence coming from the claustrum to the striate cortex. This conclusion came from the reduced end-stopping observed in striate neurons after the claustrum on one side had been ablated by an injection of kainic acid (Sherk and LeVay, 1983). The present results do little to support this interpretation, although the sample differed in that it was mainly confined to cells with receptive fields within 5^{o} of the area centralis. Using electrical stimulation to identify claustral-driven cells, few were found in lamina 2/3 while those in the other laminae showed little evidence of even moderate end-zone inhibition. This absence seemed consistent with

the fact that 7Ø% of the claustral-driven cells were C cells, a group which in the striate cortex seldom displays more than weak end zone inhibition (Henry et al., 1983). Two distinctive response features (Creutzfeldt et al., 198Ø; Sherk and LeVay, 1981) have been accorded to claustral cells - firstly, most have a preference for very long stimuli (the optimal stimulus is commonly greater than 3Ø^{o} in length), and then direction selectivity is much less common than in striate neurons. The preference for very long stimuli was not reflected in the responses of the sample of claustral-driven striate neurons. The mean optimal stimulus length (the shortest bar to produce the maximum response) was around 2^{o} for S cells and 2.3^{o} for C cells and the longest optimal stimulus for any cell was 6.2^{o}. The contribution coming from the claustrum, therefore, does not appear to influence the length of excitatory regions in the receptive fields of striate neurons.

The results from this study seem to run against the possibility that the claustral input is transposed, via an interneuron, to contribute to the inhibitory end-zones. Not only was such inhibition rare in the sample of claustral-driven cells but, even when this type of striate neuron occurred in the general population, the activity profile in the inhibitory end-zone did not reflect that of the claustral cell. Compared to the long stimuli needed to produce a maximum response from claustral cells, short stimuli produce maximum inhibition from the end-zone striate neurons. For example, in a sample taken from an

earlier study (unpublished results; Bullier et al., 1982; Henry et al., 1983) the length of the stimulus producing maximum inhibition, as it extended across the excitatory region and into the inhibitory end-zones, averaged only 2.25° (range: 0.9° to 4.2°) for 31 S cells and 1.8 (range: 1.2° to 2.4°) for two C cells. The restricted number of C cells resulted from the rarity of end-zone inhibition in this type in the striate cortex. The claustral cell, with a preference for long stimuli therefore, appears to be an inappropriate precursor for either inhibitory or excitatory regions in the small receptive fields of striate neurons. The possibility should now be considered that these claustral cells project elsewhere and that the striate cortex receives an input from those claustral cells with a preference for short stimulus (Creutzfeldt et al, 1980).

The second distinctive feature of claustral cells, the low level of directional dependence, may find expression in the responses of recipient striate neurons. The greatest potential for a claustral influence occurs in lamina 4 where claustral-driven cells display significantly weaker direction selectivity than their counterparts in the general population. The question then arises as to why the claustral cells lack direction selectivity and convey this feature on to their recipient cells in the cortex. A sensitivity to movement rather than its direction seems to be significant in the claustro-striate loop in a system that may be more involved in the detection than the direction of movement.

CHAPTER 6

DISTRIBUTION OF TRANSCALLOSAL CELLS IN THE AREAS 17, 18 AND 19 OF THE CAT

INTRODUCTION

The splenium of the corpus callosum has long been regarded as a conduit to bring signals from the visual cortex of one hemisphere into contact with those in the other. Each hemisphere carries a duplicated image of the contralateral hemifield of visual space and the axons passing in the corpus callosum arise from, and terminate on, cells that lie close to the cortical representation of the zero vertical meridian. In the primary cortical areas of the cat, the greatest concentration of callosal cells and axon terminals occurs at the common border of areas 17 and 18, at the point of representation of the vertical meridian of the visual field. In more laterally placed visual cortical areas, such as Area 19, callosal cells are more dispersed and there is a weaker concentration of vertical meridian cells (Segraves and Rosenquist, 1982). Nonetheless, the high incidence of callosal cells representing the vertical meridian has fostered the belief that transcallosal signals act to unite the neural representation of the two visual hemifields.

Using the tracer, HRP Segraves and Rosenquist (1982), estimated the spread of the transcallosal pathway away from the representation of the vertical midline in a number of regions in the visual cortex. The laminar distribution of transcallosal cells was also determined in this study. Similar aspects of the topography of the transcallosal pathway were examined with the aid of the tracer WGA-HRP, in the present study, but the experiment was designed to

ensure a finer and more detailed analysis of the cellular distribution. This more refined evaluation was needed to see how closely the transcallosal cells aligned with the terminal pattern known to hold for afferent axons of cells in the brisk transient (BT or Y) and the brisk sustained (BS or X) streams coming from the LGN.

The AIM of the present study

This study evolved from the investigation of the distribution of transcallosal cells in lamina 6 of the striate cortex. It was undertaken partly to provide information on the disposition of callosal cells in lamina 6 but also to obtain on more detailed account of the spread of transcallosal cells away from the representation of the vertical meridian in Areas 17, 18 and 19 and also of their laminar distributions in these areas. Information was also sought on the tracer patterns following the anterograde filling of terminals of transcallosal cells.

METHODS

The study used 6 cats, about 12 months old and weighing 2.Ø-3.5kg. In general, the methods are similar to that of used in Chapter 1. The tracer WGA-HRP, was injected (1ul of 5%) into the lateral convolution of the contralateral cortex. After 48hrs survival, the animal was given an overdose of Nembutal (i.v.) and, when deeply anaesthetised, was perfused through the ascending aorta. Following the perfusion, the brain was removed and allowed to sink in a solution of 3Ø% sucrose for 24-48h before sectioning at 6Øum on a freezing microtome. Sections were reacted with tetramethylbenzidine (TMB) (Mesulam 1978) (see Chapter 1), mounted and coverslipped before being examined microscopically. Representative slides were then stained with neutral red to distinguish cytoarchitectonic boundaries and laminae, which were identified from the criteria of Henry et al. (1979).

RESULTS

Distribution of transcallosal cells

In each animal an extensive injection of WGA-HRP was made into the contralateral cortex on the convolution of the lateral gyrus (HC: AP -3.Ø). At this level, the injected tracer spread throughout most of Areas 17 and 18 and some of Area 19 (cf. Segraves and Rosenquist, 1982). The resulting transport of tracer is shown for a section in the photomicrograph of Fig. 1 where transcallosally transported material in cells appears as dark spots and terminals as a dark meshwork. Figure 1 shows that the labelling of both cells and terminals was concentrated around zones representing the vertical meridian. The labelling was not confined to these regions (see also Segraves and Rosenquist, 1982), however, and extended into centre of each area.

Figure 2A quantifies a typical spread of retrogradely labelled cells observed in one animal. Labelled cell counts from 4 neighbouring histological sections, were made in Ø.25 mm wide strips that approximately paralleled the 17/18 and 19 border lines. The density of labelled cells in each strip is expressed as a percentage of the total number of labelled cells in the area. The decline in labelled cell density away from the border representing the vertical meridian was greater in Areas 17 than in areas 18 and 19. This difference was even more pronounced when cortical distance was converted into retinotopic projection lines (Harvey, 1978). When this is done the callosal

Fig. 1: A montage of the Areas 17, 18 and 19 prepared from a non-counter stained coronal section in which the tracer, WGA-HRP is stained with TMB. Labelled cells (dark spots) and terminals (dark meshwork) result from the transport of tracer after injection into the contralateral visual cortex. The greatest concentration of labelled cells and terminals is in the laminae 3/4 border zone at the junction of Areas 17 and 18. Scale bar 500µm.

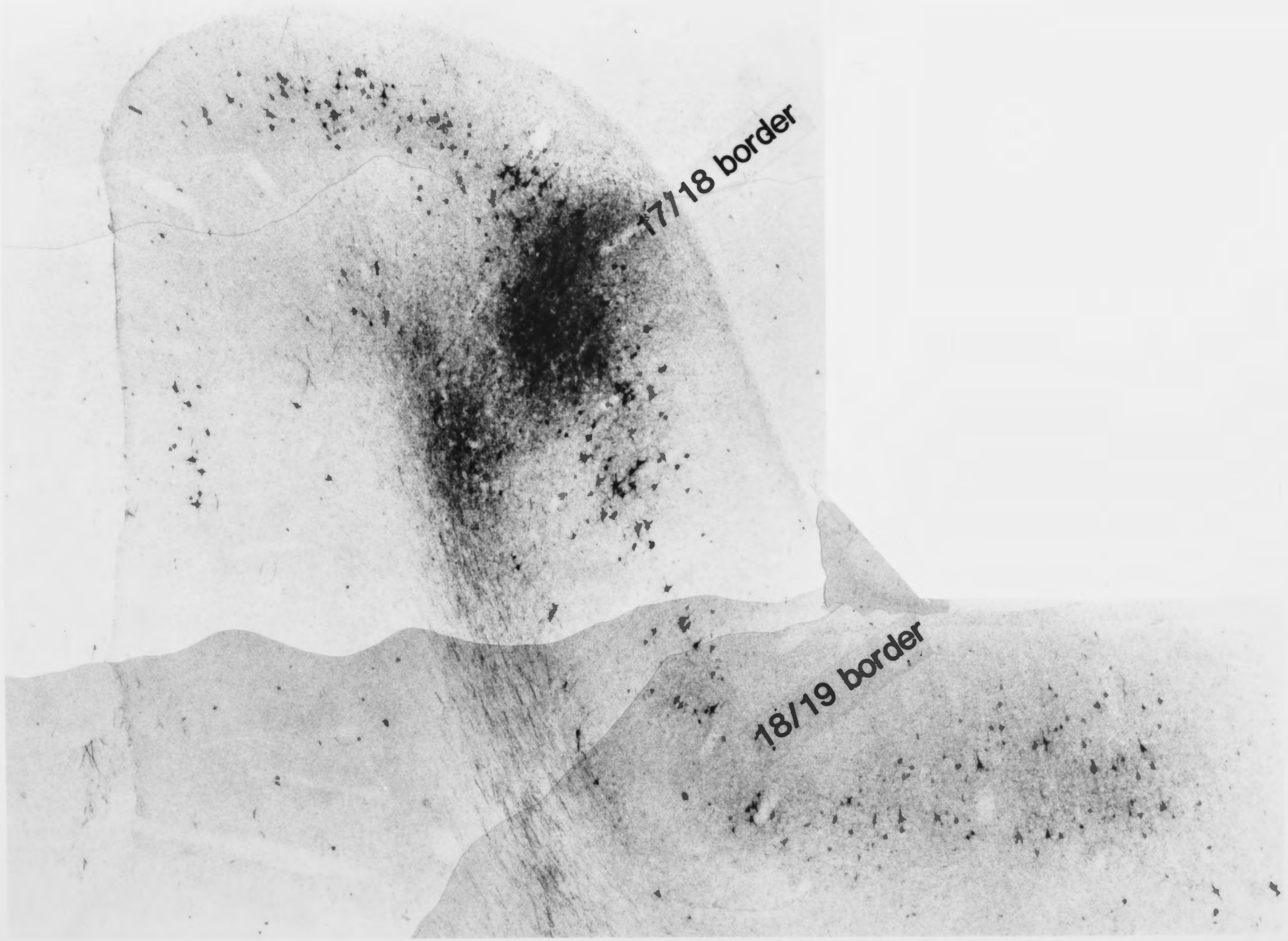
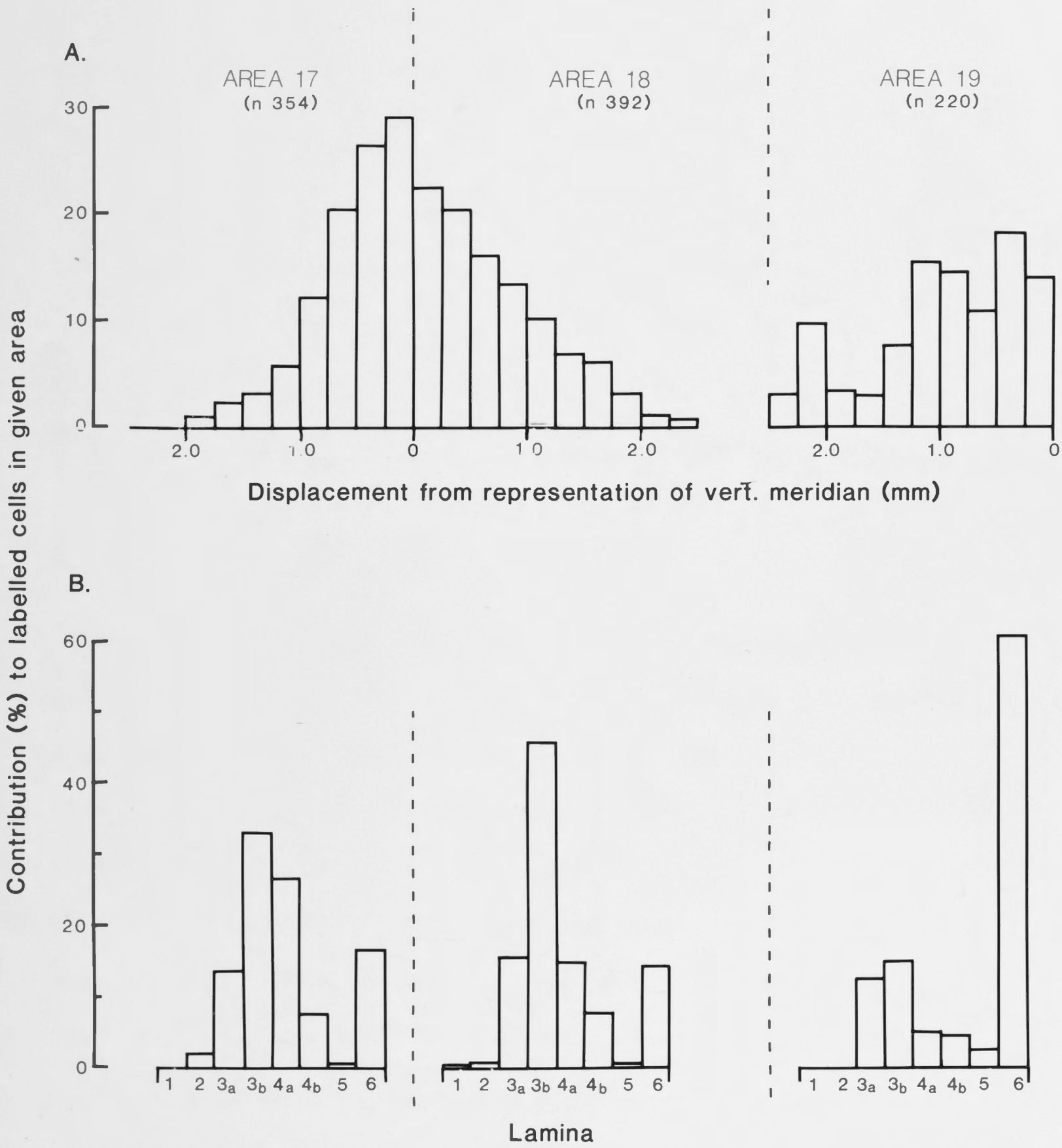

17/18 border
18/19 border

Fig. 2: The distribution of labelled cells, expressed as percentage contribution to the populations in Areas 17, 18 and 19, following the injection of HRP into the contralateral visual cortex. A. Shows the lateral spread of labelled cells away from the cortical representation of the vertical meridian in each area. B. Shows the laminar distribution of labelled cells in each area.


A.
AREA 17
(n 354)
AREA 18
(n 392)
AREA 19
(n 220)
30
20
10
0
2.0
1.0
0
1.0
2.0
2.0
1.0
0
Displacement from representation of vert. meridian (mm)
B.
Contribution (%) to labelled cells in given area
60
40
20
0
1 2 3a 3b 4a 4b 5 6
1 2 3a 3b 4a 4b 5 6
1 2 3a 3b 4a 4b 5 6
Lamina

connections in Areas 18 and 19 extended to cells representing eccentricities of more than 10° while the spread in Areas 17 was confined to cells representing visual space within 1° of the area centralis.

The distribution of labelled terminals, also apparent in the section reproduced in Fig. 1, covered much the same area as that of labelled cells although the filled terminals were more concentrated about the Areas 17/18 border. As a result, their decline in density into areas 17 and 18 was sharper than in the pattern observed for labelled cells in Fig. 2A.

The laminar distribution of transcallosal cells was reported in an earlier study (Segraves and Rosenquist, 1982) but, the need for a more detailed analysis, particularly of lamina 4 where BS and BT streams enter at different levels, prompted the construction of Fig. 2B. In these distribution histograms for Areas 17, 18 and 19, lamina 3 and 4 have each been divided to create two equal sublaminae. In the two more medial areas (17 and 18) the labelled cells congregated around the lamina 3/4 border, while in Area 19 there was a predominance of labelled cells in lamina 6. In terms of whole laminae these results are reasonably consistent with those of Segraves and Rosenquist (1982) with the exception that a higher proportion of callosal cells in lamina 6 of all areas is found than reported in the earlier study.

DISCUSSION

The recorded distribution of callosal cells revealed by the transcallosal transport of HRP confirms the earlier observation of Segraves and Rosenquist (1982), that these cells congregate around cortical zones representing the vertical meridian. Confinement to this zone, however, relaxed in the more laterally-positioned areas of the visual cortex. As a result, the spread of callosal cells away from the representation of the vertical meridian is greatest in Area 19 and least in Area 17. This disparity in topographical representation, particularly between Area 17 and the other two areas, is great enough to suggest that the callosal cells in each region may be contributing to different aspects of binocular vision and justify the search for such distinctions.

The relatively large representation in the callosal cells of Area 18, indicates that a sizable portion of the transcallosal pathway may have an association with BT or Y stream coming from the LGN. Most, if not all the cells in Area 18 receive an input from BT cells of the LGN (Harvey, 198Øb) and this must be translated to a significant level of BT information in the transcallosal pathway.

A BT stream bias in the transcallosal pathway may be reflected in the input of callosal cells in to Area 17. Thus, while both BS and BT streams enter Area 17, most callosal cells were found in the region of termination of the BT stream and only 1Ø% in lamina 4B, the point of termination of the BS stream (Bullier and Henry, 1979b).

The marked alteration in the laminar distribution of callosal cells in passing from Area 17 to Area 19, such that the level of predominance changes from the supragranular to the infragranular laminae, follows a similar pattern to that of ipsilateral cortico-cortical projections (Bullier et al., 1984). The reasons for this laminar shift are yet to be resolved in the ipsilateral projection itself (considered in Chapter 4), and its occurrence in the contralateral pathways adds a new dimension to the discussion.

CHAPTER 7

THE FUNCTIONAL INFLUENCE OF THE TRANSCALLOSAL PATHWAY OF THE CAT

INTRODUCTION

There is an extensive literature on the contribution of the transcallosal pathway to visual perception. The present study concentrates on the nature of binocular interaction that applies to signals passing through this pathway and on their specific contribution to the interpretation of visual depth.

Taking a behavioural approach to study of depth dependent signals in the transcallosal pathway, Blakemore and Mitchell (197Ø), examined the stereoscopic vision of a subject with a split callosum and found that while stereopsis was present 4^{o} or 5^{o} into the periphery it was absent along the medial saggital plane. In a companion study of another subject with a split chiasm (Blakemore, 197Ø), stereopsis was found for objects on the vertical meridian, but because of the accompanying bitemporal hemianopia, the appreciation of depth was confined to objects nearer than the point of fixation. The split callosal subject of this study does not appear to have been tested for the fine stereopsis, that could arise from the overlap in the decussation pattern of the nasal and temporal hemifields. Assuming, that the subject possessed this faculty, Bishop and Henry (1971) proposed that fine stereopsis for objects on the vertical meridian was mediated through the chiasm and that coarse or global stereopsis was achieved through the corpus callosum.

Subsequent to the proposal that fine stereopsis

depended upon the overlap in the two retinal hemifields there has been a more detailed analysis of retinal ganglion cells contributing to the overlap in the cat. From earlier data (Kirk et al., 1976), Levick (1977) plotted the retinal distribution of brisk sustained (BS) and brisk transient (BT) ganglion cells in the region of the vertical meridian. In broad terms, BS cells are equivalent to X cells (Enroth-Cugell and Robson, 1966) and BT cells, to Y cells. It was found that the line, where cells with crossed and uncrossed projections occurred in equal proportions, did not correspond to the two populations. The 50% cross over point was more temporal on the retina for BT than BS cells. Levick proposed, from these differences in overlap, that the BT system might provide a means for extending the range of midline stereopsis closer to the animal than that provided by the BS system. In terms of the Bishop and Henry (1971) suggestion, BS cells would be primarily responsible for fine stereopsis while BT cells would act alone in achieving crossed-disparity, coarse stereopsis. The natural extension of these concepts is that the transcallosal pathway would be associated closely with the BT stream of the primary visual pathway.

The AIM of the present study

The present study sought evidence of stream dependent characteristics in the visual response patterns of the cat striate neurons contributing to the transcallosal projection. Extracellular responses of single cells were used to assess receptive field properties in cells whose transcallosal links were established from responses to electrical stimulation in the contralateral hemisphere. The properties of cells contributing to and receiving from the transcallosal pathway were then evaluated to determine its relationship to BS and BT geniculo-cortical streams and thence to fine or coarse stereopsis.

METHODS

Experiments were carried out on cats which ranged in weight from 2.5 to 3.5kg. The methods employed were similar to that in Chapter 3, with the major difference being in the location of the stimulating electrodes.

In this experiment, the stimulating electrodes were positioned as follows: one in the contralateral visual cortex (CV), near the Area 17/18 border, at HC coordinates AP -3, ML 1.5; two in the corpus callosum (CC) at AP 3 and 5; ML Ø and one in or just above the ipsilateral LGN (OR_1 at AP 6.8, ML 9.Ø. The final position for the stimulating electrode at CV was determined by advancing a multi-unit recording electrode (with a large exposed tungsten tip) until units were encountered which were in retinotopic correspondence with those in the recording electrode. The CV electrode was positioned at this location. The final positions of the CC and OR_1 electrodes were set from the maximum level of multiunit activity produced by a flashing visual stimulus.

The visual responses of cells with a demonstrable callosal link were classified in S, C, B and their corresponding H categories according to criteria established earlier (Henry, 1977; Henry et al., 1978). The responses of selected cells were subjected to a computer-controlled quantitative analysis to determine if the receptive field properties of callosal cells possessed any distinctive features.

RESULTS

The sample

Transcallosal cells were recorded extracellularly from two cortical areas. One, where the larger group of cells was recorded, was at the border between Areas 17 and 18, and the other was at the lateral border of Area 19. In each cell, the link with the opposite hemisphere was identified from its response to electrical stimulation either in the contralateral visual hemisphere (CV) or in the corpus callosum (CC).

At the Area 17/18 border, 2Ø3 cells responded to stimulation in the callosal pathway, (165 were driven orthodromically and 38, antidromically). About three quarters of these cells (143 cells) were successfully located from histological reconstructions of the electrode tracks. Of these located cells 4Ø% were in Area 17, 4Ø% at the Area 17/18 border and 2Ø% in Area 18. The laminar distribution of encountered cells, found to have an anti-or ortho-dromic drive, are shown in Table 1.

The numbers in Table 1 are only descriptive of the sample and should not be regarded as an indication of the laminar distribution of callosal cells. The proportions of electrophysiologically identified callosal cells, however, did correspond well with the laminar distribution of callosal cells revealed in the tracer experiments (cf. Chapter 6).

TABLE 1

	Lamina					
Drive	3A	3B	4A	4B	5	6
Orthodromic	22	26	29	17	6	9
Antidromic	13	10	10	1	-	-

Number of Area 17/18 border cells with transcallosal drive encountered in each lamina.

Forty callosal cells were recorded also from Area 19 and, of those with an orthodromic drive, 31 were activated from CC and 14 from CV (5 cells were driven from both). In addition, 9 cells responded antidromically to stimulation at CC. Like Area 19 cells in general, most of the cells in the sample were difficult to classify from their visual responses.

CONDUCTION PROPERTIES OF THE TRANSCALLOSAL PATHWAY

The experimental arrangement of stimulating and recording electrodes to measure latencies, and so characterize the transcallosal pathway, was similar to that employed by Harvey (198Øa). The results confirm most of Harvey's findings but the larger sample permitted a more quantitative analysis of latency measurements. In particular, in the sample of 159 orthodromically driven cells in the Area 17/18 border zone, many more (1Ø8) could be driven from both CC and CV. In addition, of the 35 cells with an antidromic drive, 2Ø were also driven from both the stimulating sites. A drive from both stimulating sites (CC and CV) made it possible to calculate the conduction characteristics in the two halves of the transcallosal pathway travelling to and away from the CC stimulating site.

(a) Cells at the border of Areas 17 and 18

The distributions of latencies due to signal conduction in the two halves of the transcallosal pathway (L_{CV-CC} and L_{CC}) are plotted, for cells at the Area 17/18 border, in

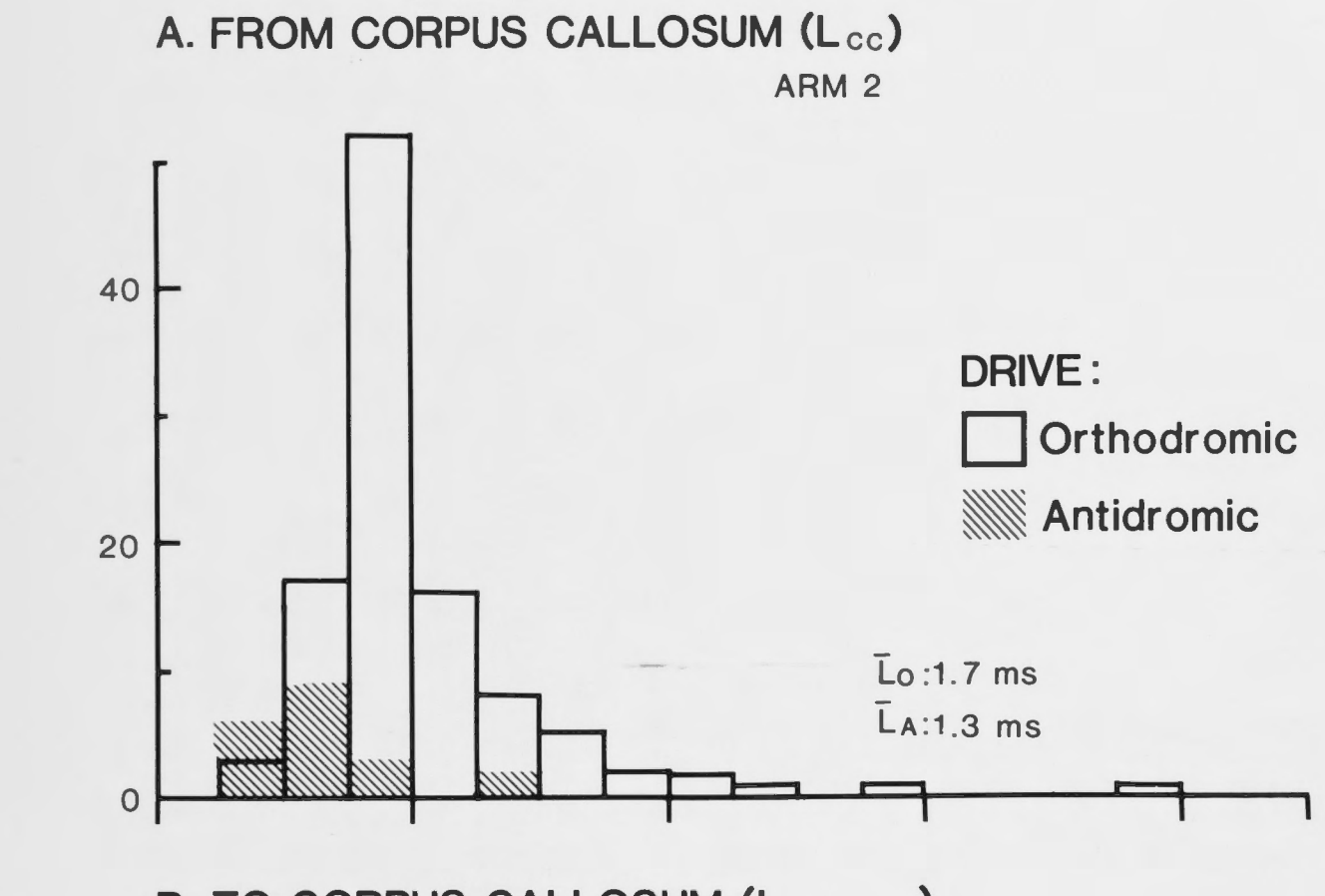
histograms A and B in Fig. 1. Cells with orthodromic and antidromic drive are presented separately. Neither histogram shows much evidence of a bimodal distribution that would indicate the existence of two streams with different conduction characteristics in the transcallosal pathway. The majority of cells (around 80%), however, occurred in a group with comparatively short orthodromic latencies (less than 2.5ms from CC).

The two histograms, A and B, in Fig. 1, show that, on average, the time taken for signals to travel from CC was longer than that to reach it. Thus, for the orthodromic response, the mean value for cells in the short-latency group (i.e. with a latency of less than 2.5ms) was for L_{CC} = 1.7ms. The corresponding value for L_{CV-CC} was 1.1ms, a value close to that in both arms for the antidromic response, L_{CC} = 1.3ms and L_{CV-CC} = 1.1ms. The longer value of the orthodromic latency, L_{CC}, may be attributed to delay at the synapse and in the narrow branches of the axon terminal. This delay was further examined in Fig. 1C by preparing a population histogram of the difference in the latencies in the two halves of the pathway ($L_{CC}-L_{(CV-CC)}$).

For identical conduction times in the two arms of the transcallosal pathway, the latency difference $L_{CC}-L_{(CV-CC)}$ would be zero. Accordingly, the values for antidromic responses, reproduced in Fig. 1C, were distributed around the zero point (mean = 0.2ms). Almost all of the orthodromic latency differences, however, possessed a positive value and their distribution assumed a bimodal

Fig. 1:. The distribution of signal conduction times of axons in the transcallosal pathway as measured from response latencies to electrical stimulation. All cells were recorded in the Area 17/18 border zone. A. Times taken to travel in ARM 2 from the corpus callosum (CC) to the recording electrode. B. Times taken to travel in ARM 1 from contralateral visual cortex (CV) to corpus callosum (CC). C. Difference in times of conduction in ARMS 1 and 2 in cells for which both measures were available. Dashed lines in C represent theoretical latency distributions for cells with mono- and di-synaptic transcallosal inputs.

RESPONSE LATENCY (Cells in area 17/18 border zone)



shape (Fig. 1C). To assist in the interpretation of this bimodality, theoretical curves were prepared for mono- and di-synaptically driven cells and added to Fig 1C (dashed lines). Postulating a monosynaptic drive, the first curve has a normal distribution whose parameters (mean: Ø.37; s.d.: Ø.3Ø; N: 66) were calculated from the latency difference values in the first peak. The second theoretical curve, based on an intracortical cell-to-cell conduction time of 1.1ms (Bullier and Henry, 1979a) and on presumed cell numbers, has the following parameters: mean: 1.47; s.d.: Ø.3Ø and N: 25). It is apparent in Fig. 1C that a number of cells had a latency difference around 1.Øms, a value which failed to meet expectations for either mono- or di-synaptically driven cells. One interpretation is that these cells are amongst those which receive their orthodromic drive via the collaterals of an efferent cell. It is noteworthy that the same proportion of each cell type (C, S and B - see below) contributed to this peak.

The predominance of the first peak in the latency distribution of Fig. 1C, indicates that a high proportion of the encountered cells receive a direct input from the contralateral cortex. Many of these cells also receive a short-latency, presumably monosynaptic input from the LGN (OR_1). The distribution histogram of OR_1 latencies for callosal cells (not included in present report) was similar to that observed for other populations of striate neurons (Boyapati and Henry, 1985; Bullier and Henry, 1979a). It had a large short-latency peak, between 1.Øms and 2.Øms followed by a secondary peak, between 2.2 ms and 3.2ms. It

has been argued that this bimodal latency distribution indicates the presence of separate populations of mono- and di-synaptically driven cells, respectively. Of the cells judged to be directly driven from the LGN, many (14/2Ø) occurred in the first peak in Fig. 1C and, so appeared to be monosynaptically driven also from the corpus callosum.

(b) Cells at the lateral border of Area 19

The histograms of Fig. 2 show the distributions of response latencies recorded in the two halves of the transcallosal pathway (L_{CV-CC} and L_{CC}) linking contralateral CV (Area 17/18 border) with Area 19. These histograms have a similar form to the equivalent ones for the Area 17/18 border region. The mean values for cells with orthodromic latencies less that 2.5ms was L_{CC} = 1.9ms and L_{CV-CC} = 1.4ms - two values that only differed by Ø.2ms from the corresponding parameters in the Area 17/18 sample. The mean latency for antidromic responses from CC was 2.Øms, which, despite the absence of synaptic delay, is similar to the figure for orthodromic responses. With such a small sample, however, there is no justification for concluding that the signal conduction rates differ in the two directions.

Receptive fields of transcallosal cells

The full spectrum of receptive field types was encountered in the sample of 165 orthodromically-driven callosal cells. The relative numbers of each cell type are shown in Table 2. Approximately, 4Ø% of the sample,

Fig. 2: Distributions of signal conduction times in two arms of transcallosal pathway (L_{CC} and L_{CV-CC}) for cells in Area 19. Conduction times measured from response latencies to electrical stimulation at the corpus callosum (CC) and contralateral visual cortex (CV).

RESPONSE LATENCY (Cells in area 19)

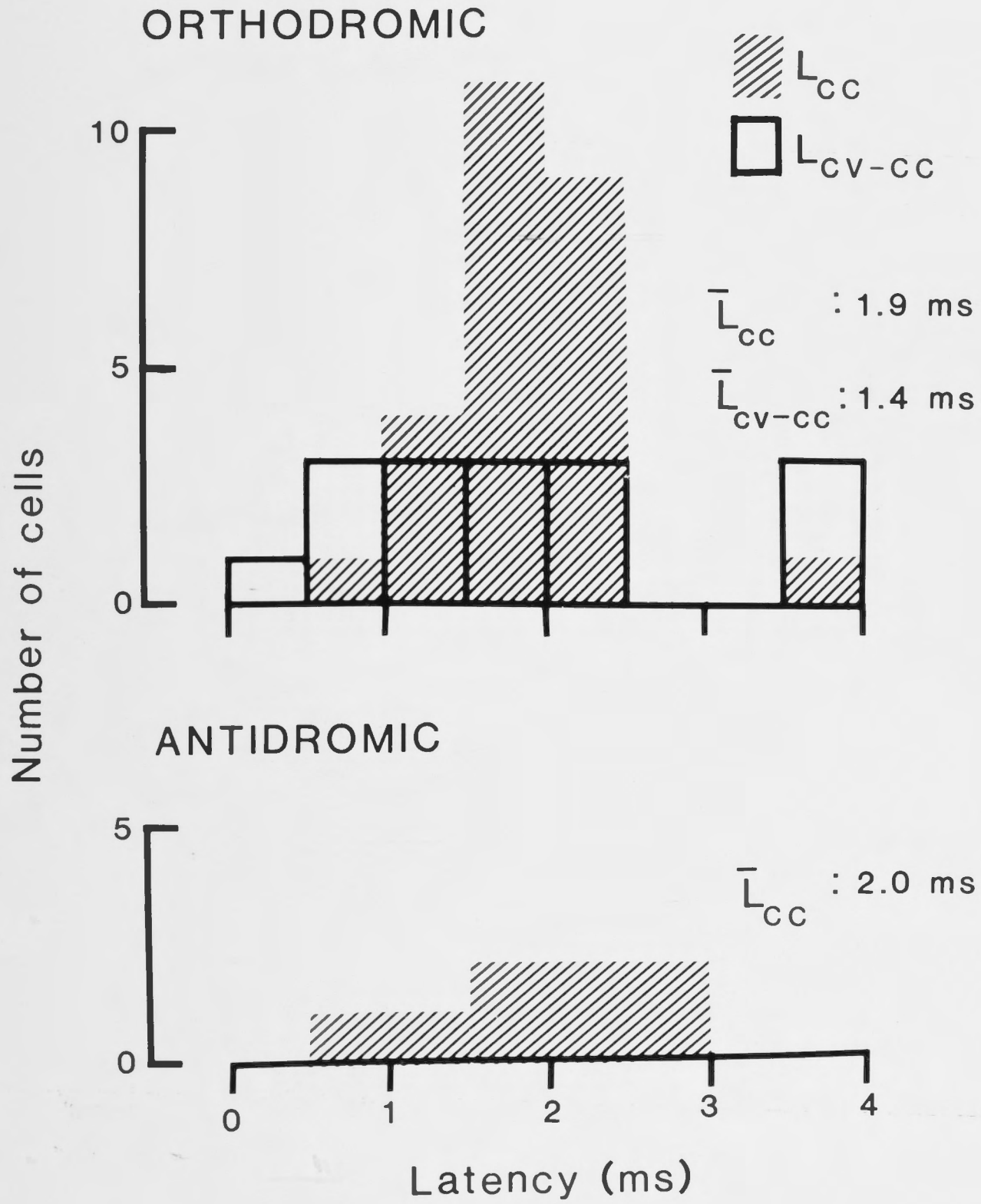


TABLE 2

Drive	S	S_H	C	C_H	B	B_H	NO
Orthodromic	22.7	7.5	48.0	2.5	15.1	1.6	2.6
Antidromic	15.0	10.0	58.0	-	20.0	-	-

NO: Non oriented receptive field

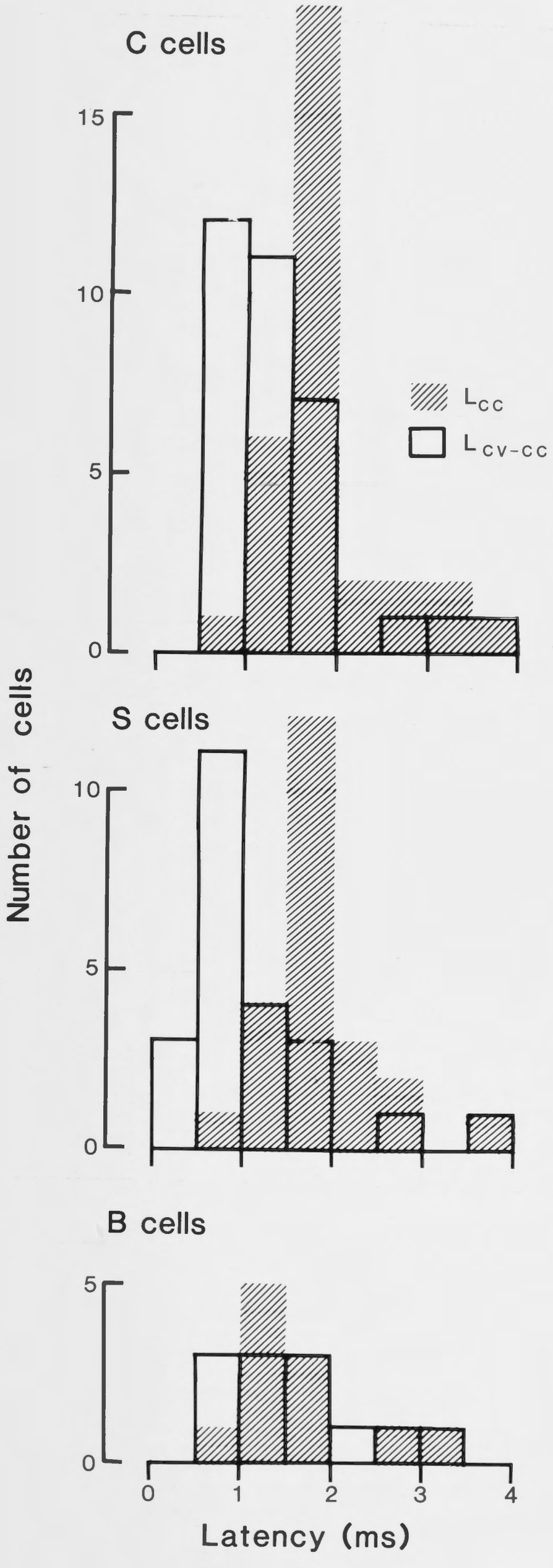
Classified Area 17/18 border cells, recorded as % of sample, in the transcallosal pathway

principally made up from cells in lamina 3, were excluded from Table 2 because they could not be classified unambiguously. Similar percentages were found for cell types amongst the 38 antidromically driven cells.

From Table 2, about 50% of the cells with either anti- or ortho-dromic drive belonged to the C category. The high probability that this class of cells receives a primary input from the BT stream, prompted a search for other signs of BT stream affiliation amongst callosal cells. Examining the number of callosal cells in the primary regions where the BS and BT streams terminate, it was found that around 90% of both S (12/13) and C (28/32) were in the region of BT stream termination (i.e. laminae 3B and 4A in Area 17 and in Area 18). A small number of cells were found in lamina 4B in the region of termination of the BS stream, however, and transcallosal cells were found also in the B category -a group that appears to receive a BS input (Henry et al., 1983). Some cells of the BS stream, therefore, appear to contribute to the transcallosal pathway.

The latencies to electrical stimulation at CC and CV showed no evidence of class distinctions associated with different cell types. The histograms in Fig. 3, which show the latencies for signal conduction in the two halves of the transcallosal pathway for C, S and B cells, reveal a similarity amongst the three classes. Each histogram in turn, resembles the two distributions recorded in Figs. 1A and B, where the same parameters are plotted for the total sample of callosal cells. No distinctive class features

Fig. 3: Distributions of signal conduction times in two arms of transcallosal pathway (L_{CC} and L_{CV-CC}) for different types of cells in the Area 17/18 border zone.


C cells
15
10
5
0
L_{cc}
L_{cv-cc}
Number of cells
S cells
10
5
0
B cells
5
0
0
1
2
3
4
Latency (ms)

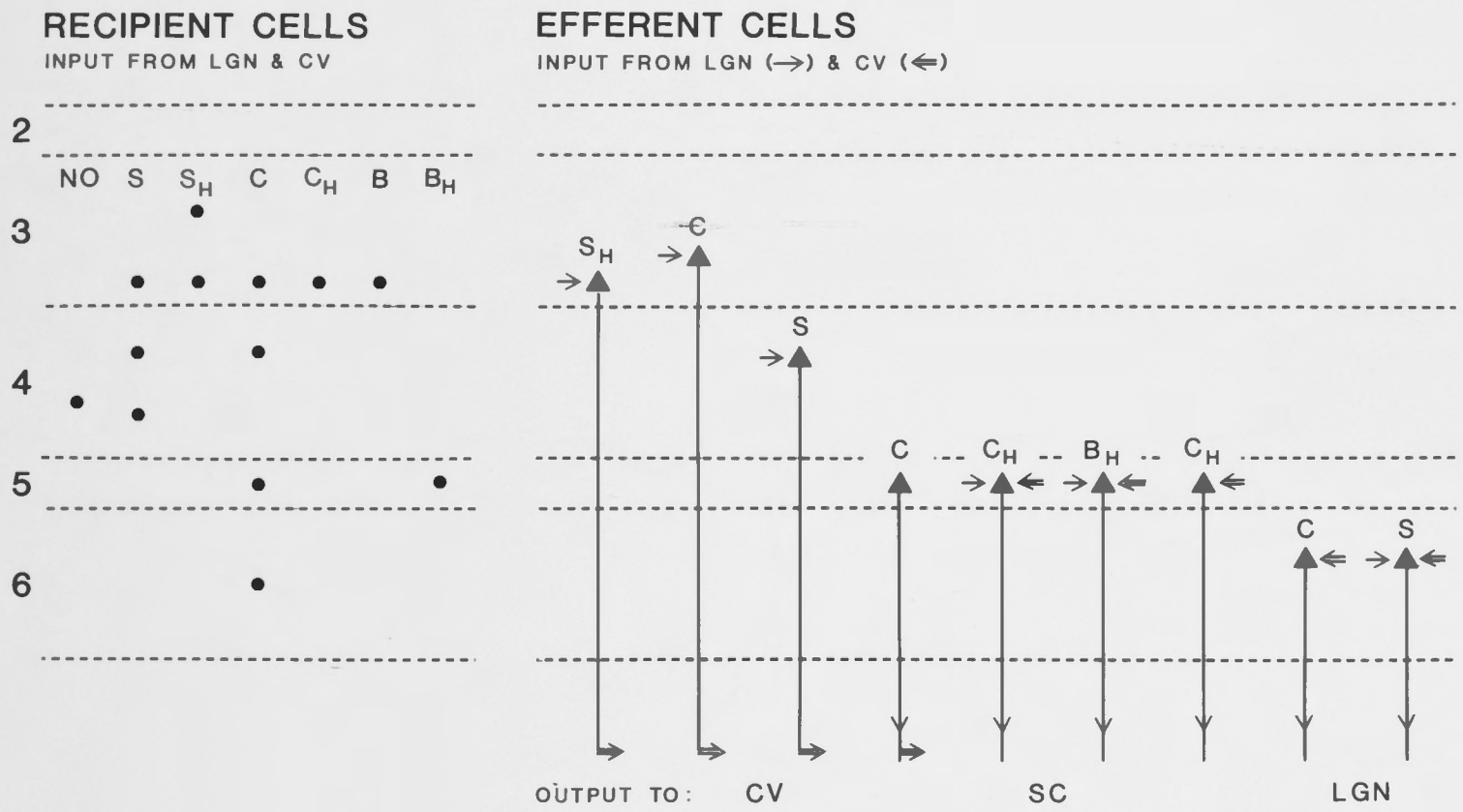
emerged from the histograms in Fig. 3.

Neural circuity of transcallosal cells

With stimulating electrodes located just above the LGN (OR_1), as well as in the opposite hemisphere, and the capability to detect both ortho- and anti-dromic responses, it was possible to draw a number of connectivity diagrams for a variety of cells occurring in different laminae in the Area 17/18 border region. This diagram appears in Fig. 4 where the filled circles, to the left, represent examples of recipient cells driven orthodromically from both the primary afferent (OR_1) and the transcallosal (CV) pathway. A wide variety of cell types possessed the double orthodromic input.

Efferent cells, represented by triangles to the right in Fig. 4, were antidromically driven through an efferent axon and, therefore, were probably pyramidal cells. Many also received an orthodromic drive, indicated by the horizontal arrows, from one or both the stimulating sites (CC/CV or OR_1). The destination of cells antidromically driven from OR_1 was assigned from the understanding that cells in lamina 5 project principally to the superior colliculus (SC), via a path that skirts the LGN, while those in lamina 6 pass into the LGN (Gilbert and Kelly, 1975). Cells driven antidromically from the contralateral hemisphere were found mainly in laminae 4A or 3B, although one C cell in lamina 5 responded antidromically to stimulation at both CV and OR_1 and thus appeared to have a bifurcating axon that projected both to the SC and the

Fig. 4: Schematic representation of cells whose connectivity has been established from occurrence of ortho- or anti-dromic drives from stimulating electrodes in the transcallosal pathway (CC or CV) or in the optic radiations just above the LGN (OR_1). Input, from ipsi- or contra-lateral hemispheres, identified from orthodromic drive; output, from antidromic drive. NO: non oriented receptive field; S, C, B and H: class of cell as designated in text. CV: contralateral visual cortex; SC: superior colliculus; LGN: lateral geniculate nucleus.


RECIPIENT CELLS
INPUT FROM LGN & CV
2
NO
S
S_H
C
C_H
B
B_H
3
4
5
6
EFFERENT CELLS
INPUT FROM LGN (→) & CV (⇐)
S_H
C
S
C
C_H
B_H
C_H
C
S
OUTPUT TO:
CV
SC
LGN

contralateral cortex.

The visual responses of callosal neurons

No outstanding distinctions were observed in the visual responses of callosal cells although it is noteworthy that so many callosal cells were located along the lamina 3/4 border zone of Area 17 and in Area 18. These are principal regions of termination of the BT stream coming from the LGN and the receptive field properties of many of the cells reflected this association. Thus, the majority of recorded S cells and all of the C cells, in contradistinction to the B cells, had the relatively large receptive fields characteristic of cells receiving a BT stream input. In cortical cells in general, however, few other response properties appear to be stream dependent, and likewise no distinctive features were found in callosal cells for properties such as direction selectivity, orientation specificity or stimulus velocity dependence. Even for binocularity, where callosal cells might have been expected to show some unique variation, the ocular dominance distribution for the sample was similar to that of the general population. Of the 151 cells given ocular dominance ratings from 1 to 7 (Hubel and Wiesel, 1962), 50% were strongly binocular (groups 3, 4 and 5) while 33% were monocular and were placed in groups 1 and 7.

DISCUSSION

The present study has provided information concerning the cortical distribution of transcallosal cells, the conduction properties of their axons, and the nature of their visual responses. Attention will be directed towards these areas in the following interpretation of the results.

Conduction properties of transcallosal axons

There are advantages in using the transcallosal pathway as a line for studying the conduction characteristics of axons transmitting impulses initiated by electrical stimulation. Since the CV stimulating site is at the end of the pathway and the CC is in the middle, it is possible to compare conduction times in the two halves of the pathway. The equivalence of the distance in each half is demonstrated by the similar time taken to conduct antidromic responses (mean difference in the two halves: L_{CC}-L_{CV-CC} = Ø.2ms). By contrast, unequal conducting times in the two halves do appear with orthodromic responses because of the synaptic delay incurred in the second half of the pathway. In a high proportion of orthodromically driven cells, however, the delay appears minimal and suggests that the method can effectively isolate directly-driven cells. Thus, the group of cells in the first peak in Fig. 1C, which have a mean difference of conduction time in the two halves of Ø.37ms, is likely to receive a direct callosal drive. Since most of these cells were in the lamina 3/4 border zone they are also likely to

be driven directly from the LGN.

For the second and smaller group of cells with differences in conduction times in the two halves of around 1.Øms (see Fig. 1C) the delay in the second half is too long for one synapse and too short for two (synaptic delay + cell to cell conduction time = Ø.4 + 1.1ms). The time difference is consistent, however, with a conduction time of 1.1ms in the cortex (Bullier and Henry, 1979a). The possibility arises, therefore, that such signals may pass via a collateral axon of an efferent cell. If this is, in fact, an artifactual form of signal transmission, it does not appear to be associated exclusively with a particular cell type since a similar proportion of each class contributed to the group. The majority of these cells (12/14) were encountered high in the cortex in laminae 3 or 4a.

It was hoped initially that the level of heterogeneity of conduction times in the population of callosal axons would provide evidence for or against multiple streams in the callosal pathway. The results, however, failed to give a clear picture of sub-populations in the spectrum of axons. In particular, there was no evidence of bimodality in the dastribution of latencies over the full pathway from CV (not included amongst figures) or for either of its halves (CC or CV-CC). The temptation to regard a unimodal latency distribution as evidence for a single stream must be tempered, however, by the observation of Cleland et al. (1976), who showed that the conduction latencies of axons

in two streams in the geniculo-striate pathway (BT and BS) merge to form a single peak. In this case, the conduction times of geniculo-striate axons were obtained by stimulating the striate cortex and measuring antidromic latencies in identified LGN neurons. This pathway is similar in distance and also axonal conduction velocity to those in the first half of the transcallosal pathway (compare Fig. 7 of Cleland et al. (1976) with Fig. 1B). Despite the dual streaming in the geniculo-striate pathway, the unimodal distribution of axonal conduction times (Cleland et al., 1976) is almost identical to that for the first half of the transcallosal pathway. The single peak in Fig. 1B, therefore, while consistent with a single transcallosal stream, is not inconsistent with a dual one.

In agreement with Harvey (1980a), electrical stimulation in the present study has revealed a high proportion of comparatively fast conducting axons in the transcallosal pathway. These axons could maintain or meet, as the case may be, the fast conducting stream composed of the axons of BT cells of the geniculo-cortical pathway. The existence of such a stream is also in accord with the laminar distribution of cells labelled by transcallosal tracer. However, neither the experiment involving electrical stimulation nor the one with the transcallosal transport of label, has completely excluded the possibility of a second transcallosal path that works in association with the BS stream of the geniculo-cortical input.

Transcallosal cell types

The use of anti- and ortho-dromic responses to identify callosal cells led to the examination of two populations - one, of contributing cells and the other, of recipient cells (see Fig. 4). The experimental samples of these two groups, although differing in size (165 to 38 cells), contained essentially the same proportions of the different cell types. In both samples, about 50% of the cells were C cells while S and B cells each contributed around 20%. These percentages are unlikely to be biased by sampling a disproportionately large number of cells in Area 18. The tracks of all electrodes started in Area 17, entered the border region and were terminated immediately on entering Area 18. Likewise, C cells do not appear to be inherently more responsive to orthodromic stimulation than other types and were not predominant in samples taken after electrical stimulation in the optic radiations (Bullier and Henry, 1979a).

Evidence from earlier studies (Bullier and Henry, 1979b; Tanaka, 1983) indicates that C cells have a primary input coming from BT cells in the LGN, in contrast to B cells which receive from BS cells (Henry et al., 1983). The S cells, on the other hand, may be sub-divided according to whether their input comes either from the BT or the BS stream. Most of the callosal cells belonging to the S family are likely, from their distribution in the lamina 3/4 border zone and their large receptive fields, to belong to the sub group receiving a BT cell input. As many

as 75% of the callosal cells (receivers or contributors) in the Area 17/18 border zone could receive their primary input from the BT stream and the transcallosal contribution to the visual process is likely to reflect this association. In this regard, it may be significant that the experimental sectioning of the corpus callosum in the cat leads to a decline in binocularity in C cells and a subclass of S cells (Payne et al., 1984).

The possibility, advanced by Bishop and Henry (1971) (see also Harvey, 198Øa), that the corpus callosum conveys information necessary for coarse stereopsis has not been brought into dispute by the present study. Rather a number of the findings lend some support to the proposal. For example, callosal cells are located in the regions of termination of the BT axons of the geniculo-cortical pathway and signals also travel through the callosum as fast as those in the primary BT pathway. In addition, there is a high proportion of callosal cells with receptive fields that are characteristic of cortical neurons of the BT stream. The retinotopic spread of cortical callosal cells extends several degrees into the ipsilateral hemifield and in so doing parallels the spread of BT ganglion cells in the retina. All of these features point to a transcallosal link with the BT system but this does not exclude the possibility that the BS stream provides an input to callosal cells; rather it emphasises that the proportion of these cells is much lower than those receiving from the BT stream.

Whether the callosal cells, with their BT inputs, service the same region of visual space as that covered by overlapping ganglion cells remains an unanswered question. It seems an unnecessary duplication that the binocular overlapping achieved through the corpus callosum should replicate that provided by BT ganglion cells and be confined to the detection of crossed disparity for nearer objects. It has been demonstrated in human vision that coarse stereopsis arises not only from crossed but also uncrossed disparity (that is from both nearer and farther points in the medial saggital plane (Westheimer and Tanzman, 1956)). The cat also appears to have coarse stereopsis for nearer and farther points (Fox and Blake, 1971). It would be a tidy arrangement if retinal overlap of BT ganglion cells serviced crossed disparities while the callosal link accounted for uncrossed disparities. It appears for the human, however, that the transcallosal pathway carries information on both crossed and uncrossed disparities. This is reflected in the loss of ability of the split callosal subject to correctly converge or diverge as an initial response to coarse disparities of objects on the medial saggital plane (Westheimer and Mitchell, 1969). Correct convergence would have been retained if the overlap of BT ganglion cells had provided the framework for coarse stereopsis. In the cat, coarse stereopsis could be linked with activity in the transcallosal pathway and, if evidence from human psychophysics can be applied, the information transferred along this pathway relates to both crossed and uncrossed disparities.

GENERAL CONCLUSIONS

The general pattern adopted for this work has been to make paired studies of the morphology and function of a number of neural pathways associated with the striate cortex and, in particular, with the lamina 6 of this area. In the main, the cells of lamina 6 provide an exit for cortical communication with subcortical structures but recent evidence (LeVay and Sherk, 1981; Bullier et al., in preparation), and that of the present study, indicates that some lamina 6 cells project to other parts of the visual cortex.

It was a primary aim of the study to discover if regular patterns existed in the design of the different pathways arising from lamina 6. The results provide a summary of the properties of each pathway and help to separate the common from the distinctive features. Even at the point of origin in lamina 6, however, the pathways appear to arise from different cell populations. Within this sublayering, the cells contributing to the LGN are much more common and cover a wider expanse of the lamina than those of the other two pathways. The layering, although far from complete, appears to indicate that each pathway carries distinctive information. Little can be said of the functional individuality of lamina 6 cells contribution to each pathway since, in the recordings from the striate cortex, almost no cells were encountered contributing to either the claustral or the transcallosal pathways. This low encounter rate is almost certainly due

to the sparseness of these cells in lamina 6 and it may be some time before functional characterstics are uncovered in such dispersed populations.

The partial sublayering of lamina 6 stands alone as evidence of functional differentiation and, on its own allows few conclusions to be drawn. One argument developed from the lamination is the cells projecting to the LGN, because of their mid-laminar location, are likely to carry more general information than the border populations that project to the claustrum and extra-striate cortex.

As a counter to the idea of functional differentiation, however, there are a number of similarities in the neural loops passing through lamina 6. Since little information is available on the nature of the outward pathways from lamina 6, (in particular, to the claustrum and to the corpus callosum) the comparison must be confined to the reciprocal half of the loop returning to the striate cortex. For these return pathways, the most notable similarities are in the conduction properties of axons passing from the claustrum and the contralateral cortex and also in the type of cells receiving an input from these pathways. In both cases, there is a preponderance of fast conducting axons and the greatest number of recipient cells belong to the C category of striate neurons. Not only are these cells from the same class but they also occupy similar laminar positions. The cells at these locations at lamina 3/4 border and in lamina 6 are known to receive an input from the Y or brisk transient stream in the pathway

LGN. In the present comparison, the link from the LGN, should not be regarded as a returning path but more as the continuation of the primary visual input passing through the LGN.

The main conclusion, therefore, from the comparison of the claustral and transcallosal loops is that they are both heavily involved with the Y stream of the primary input. From this point of correspondence it seems that both loops may have an important role to play in movement detection, or in low resolution, pre-attentive vision. In the transcallosal pathway this association with the Y stream could translate into coarse or global stereopsis. It may be an additional aspect to the involvement with coarse stereopsis that a heavy transcallosal pathway was observed following the injection of tracer into the claustrum. It would be of interest now to discover if the properties of these axons are similar to those of the cortical transcallosal link.

It is appropriate, in considering the transcallosal pathways from cortex and claustrum, to add the inter hemispheric projections from EVA. The present study has shown that EVA has a strong link with the homotopic cortex of the contralateral hemisphere and also that this connection may pass through the corpus callosum or through the anterior commissure. These bilateral connections, plus links with the mid brain and the lateral regions of the visual cortex -the tectal receiving cortex (Dreher, 1985) -suggest that EVA may be involved in the control of

binocular eye movements. The tracer experiments conducted on EVA, although providing valuable information, were initiated principally as a control step to ensure that the area was not contaminated during the injection of tracer into the claustrum. Despite their proximity, these two structures show marked differences in their pattern of connectivity and there appears to be little interaction between the two regions.

The proposal that the claustral cells add the hypercomplex or H property to striate neuron does not receive support from the present study. The H property which has been linked traditionally with high-resolution form vision is unlikely to be derived from a nucleus that has such strong affiliations with the Y system. Moreover, an association with the H property also appeared to be ruled out by the low incidence of the H property in claustral driven cells and also in the disparity between the dimensions of claustral cell receptive fields and end-zone inhibitory regions of hypercomplex cells.

The connections that the claustrum makes with such a wide variety of regions in the visual cortex fosters the view that successive steps in visual processing are influenced by information available in the claustrum. The nature of claustral interaction is unresolved although there have been suggestions that there is no cross-links between zones of different sensory modality in the nucleus. It would be surprising from the point of view of topographical design, however, if signals from different

sensory systems came together in the claustrum and then failed to interact. For the moment, the possibility should be retained that the claustrum is a centre where visual processes, more than likely involved with pre-attentive vision, interact with other forms of sensory information.

As hinted above, the projection from lamina 6 to the PGN and the LGN forms one half of an open loop which is constantly receiving inputs from the eye. In this arrangement, the contribution of the corticofugal path remains a mystery although the present study has helped in the interpretation of the proposal that S cells project to the LGN and C cells to the PGN. If it is ultimately confirmed beyond doubt that the S cells provide an input to the LGN then the influence of this contribution will still require evaluation. Evidence of the S cell response specificity remains undetected in either excitatory or inhibitory components of the LGN cell. In the course of the experiments on the LGN a thorough search was undertaken for an excitatory input from the cortex. It was reasoned that the corticofugal pathway could act as a delay line for the recycling of cortical messages to replace incoming signals lost during saccadic eye movements. The absence of any signs of an excitatory infuence from the cortex, however, prevented further exploration of this idea. There is a little more encouragement in the responses of PGN cells which often do appear to reflect the response pattern of C cells. The negative aspect of this relationship, however, lies in the absence of consistent reponses in PGN cells.

Much more information is required before the picture is complete on the organization of lamina 6. The present study has added to the available material on the structure and function of lamina 6 in the striate cortex but generalizations will, of necessity, depend on future studies in extra-striate visual cortex. Taking the results from the striate cortex as a guide there are a number of questions to be answered in formulating the organizational rules that may hold for throughout the visual cortex. For example, does the major projection from lamina 6 always go to nucleus providing the principal afferent supply? If so is there a common purpose to these feed backs? Does the strength of the claustral link, which is comparatively weak for the striate cortex, relate to the visual role of the cortical area?. Is the strength of the transcallosal link also indicative of the area's contribution to coarse stereopsis? There are many more questions of this nature and the present study will be worthwhile if it prompts someone, somewhere to seek their solution.

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